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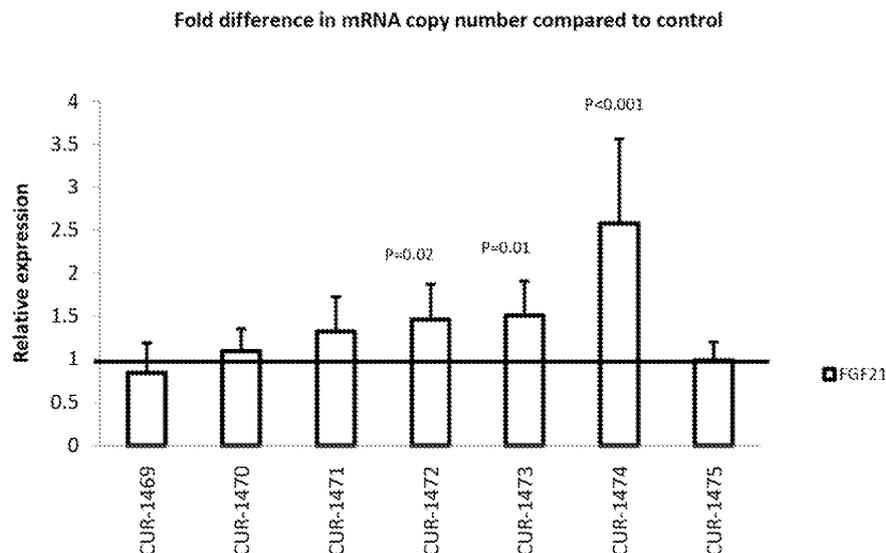
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(54) **TREATMENT OF FIBROBLAST GROWTH FACTOR 21 (FGF21) RELATED DISEASES BY INHIBITION OF NATURAL ANTISENSE TRANSCRIPT TO FGF21**

(57) The present invention relates to antisense oligonucleotides that modulate the expression of and/or function of Fibroblast growth factor 21 (FGF21), in particular, by targeting natural antisense polynucleotides of

Fibroblast growth factor 21 (FGF21). The invention also relates to the identification of these antisense oligonucleotides and their use in treating diseases and disorders associated with expression of FGF21.

**FIGURE 1**



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**Description****FIELD OF THE INVENTION**

5 **[0001]** The present application claims the priority of U.S. provisional patent application No. 61/322352 filed April 9, 2010 which is incorporated herein by reference in its entirety.

**[0002]** Embodiments of the invention comprise oligonucleotides modulating expression and/or function of FGF21 and associated molecules.

10 **BACKGROUND**

**[0003]** DNA-RNA and RNA-RNA hybridization are important to many aspects of nucleic acid function including DNA replication, transcription, and translation. Hybridization is also central to a variety of technologies that either detect a particular nucleic acid or alter its expression. Antisense nucleotides, for example, disrupt gene expression by hybridizing to target RNA, thereby interfering with RNA splicing, transcription, translation, and replication. Antisense DNA has the added feature that DNA-RNA hybrids serve as a substrate for digestion by ribonuclease H, an activity that is present in most cell types. Antisense molecules can be delivered into cells, as is the case for oligodeoxynucleotides (ODNs), or they can be expressed from endogenous genes as RNA molecules. The FDA recently approved an antisense drug, VITRAVENE™ (for treatment of cytomegalovirus retinitis), reflecting that antisense has therapeutic utility.

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**SUMMARY**

**[0004]** This Summary is provided to present a summary of the invention to briefly indicate the nature and substance of the invention. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

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**[0005]** In one embodiment, the invention provides methods for inhibiting the action of a natural antisense transcript by using antisense oligonucleotide(s) targeted to any region of the natural antisense transcript resulting in up-regulation of the corresponding sense gene. It is also contemplated herein that inhibition of the natural antisense transcript can be achieved by siRNA, ribozymes and small molecules, which are considered to be within the scope of the present invention.

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**[0006]** One embodiment provides a method of modulating function and/or expression of an FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 4246 of SEQ ID NO: 2 thereby modulating function and/or expression of the FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

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**[0007]** In an embodiment, an oligonucleotide targets a natural antisense sequence of FGF21 polynucleotides, for example, nucleotides set forth in SEQ ID NOS: 2, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 3 to 9.

**[0008]** Another embodiment provides a method of modulating function and/or expression of an FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to a reverse complement of the an antisense of the FGF21 polynucleotide; thereby modulating function and/or expression of the FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

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**[0009]** Another embodiment provides a method of modulating function and/or expression of an FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising contacting said cells or tissues with an antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to an antisense oligonucleotide to an FGF21 antisense polynucleotide; thereby modulating function and/or expression of the FGF21 polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

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**[0010]** In an embodiment, a composition comprises one or more antisense oligonucleotides which bind to sense and/or antisense FGF21 polynucleotides.

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**[0011]** In an embodiment, the oligonucleotides comprise one or more modified or substituted nucleotides.

**[0012]** In an embodiment, the oligonucleotides comprise one or more modified bonds.

**[0013]** In yet another embodiment, the modified nucleotides comprise modified bases comprising phosphorothioate, methylphosphonate, peptide nucleic acids, 2'-O-methyl, fluoro- or carbon, methylene or other locked nucleic acid (LNA) molecules. Preferably, the modified nucleotides are locked nucleic acid molecules, including  $\alpha$ -L-LNA.

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**[0014]** In an embodiment, the oligonucleotides are administered to a patient subcutaneously, intramuscularly, intravenously or intraperitoneally.

**[0015]** In an embodiment, the oligonucleotides are administered in a pharmaceutical composition. A treatment regimen comprises administering the antisense compounds at least once to patient; however, this treatment can be modified to

include multiple doses over a period of time. The treatment can be combined with one or more other types of therapies.

**[0016]** In an embodiment, the oligonucleotides are encapsulated in a liposome or attached to a carrier molecule (e.g. cholesterol, TAT peptide).

**[0017]** Other aspects are described *infra*.

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## BRIEF DESCRIPTION OF THE DRAWINGS

**[0018]** Figure 1 is a graph of real time PCR results showing the fold change + standard deviation in FGF21 mRNA after treatment of MCF-7 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Bars denoted as CUR-1469 to CUR-1475 correspond to samples treated with SEQ ID NOS: 3 to 9 respectively. Figure 2 is a graph of real time PCR results showing the fold change + standard deviation in FGF21 mRNA after treatment of HcpG2 cells with phosphorothioate oligonucleotides introduced using Lipofectamine 2000, as compared to control. Bars denoted as CUR-1469 to CUR-1475 correspond to samples treated with SEQ ID NOS: 3 to 9 respectively.

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**[0019]** Sequence Listing Description- SEQ ID NO: 1: Homo sapiens fibroblast growth factor 21 (FGF21), mRNA (NCBI Accession No.: NM\_019113); SEQ ID NO: 2: Natural FGF21 antisense sequence (Hs.69747); SEQ ID NOS: 3 to 9: Antisense oligonucleotides. \* indicates phosphorothioate bond.

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## DETAILED DESCRIPTION

**[0020]** Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. The present invention is not limited by the ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

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**[0021]** All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In an embodiment, the genes or nucleic acid sequences are human.

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### Definitions

**[0022]** The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

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**[0023]** The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

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**[0024]** As used herein, the term "mRNA" means the presently known mRNA transcript(s) of a targeted gene, and any further transcripts which may be elucidated.

**[0025]** By "antisense oligonucleotides" or "antisense compound" is meant an RNA or DNA molecule that binds to another RNA or DNA (target RNA, DNA). For example, if it is an RNA oligonucleotide it binds to another RNA target by means of RNA-RNA interactions and alters the activity of the target RNA. An antisense oligonucleotide can upregulate or downregulate expression and/or function of a particular polynucleotide. The definition is meant to include any foreign RNA or DNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such molecules include, for example, antisense RNA or DNA molecules, interference RNA (RNAi), micro RNA, decoy RNA molecules, siRNA,

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enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

5 **[0026]** In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. The term "oligonucleotide", also includes linear or circular oligomers of natural and/or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, substituted and alpha-anomeric forms thereof, peptide nucleic acids (PNA), locked nucleic acids (LNA), phosphorothioate, methylphosphonate, and the like. Oligonucleotides are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, Hoogsteen or reverse Hoogsteen types of base pairing, or the like.

10 **[0027]** The oligonucleotide may be "chimeric", that is, composed of different regions. In the context of this invention "chimeric" compounds are oligonucleotides, which contain two or more chemical regions, for example, DNA region(s), RNA region(s), PNA region(s) etc. Each chemical region is made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotides compound. These oligonucleotides typically comprise at least one region wherein the oligonucleotide is modified in order to exhibit one or more desired properties. The desired properties of the oligonucleotide include, but are not limited to, for example, to increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. Different regions of the oligonucleotide may therefore have different properties. The chimeric oligonucleotides of the present invention can be formed as mixed structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide analogs as described above.

20 **[0028]** The oligonucleotide can be composed of regions that can be linked in "register", that is, when the monomers are linked consecutively, as in native DNA, or linked via spacers. The spacers are intended to constitute a covalent "bridge" between the regions and have in preferred cases a length not exceeding about 100 carbon atoms. The spacers may carry different functionalities, for example, having positive or negative charge, carry special nucleic acid binding properties (intercalators, groove binders, toxins, fluorophors etc.), being lipophilic, inducing special secondary structures like, for example, alanine containing peptides that induce alpha-helices.

25 **[0029]** As used herein "FGF21" and "Fibroblast growth factor 21" are inclusive of all family members, mutants, alleles, fragments, species, coding and noncoding sequences, sense and antisense polynucleotide strands, etc.

30 **[0030]** As used herein, the words 'Fibroblast growth factor 21', FGF21, FGF-21, UNQ3115 and PRO10196, are considered the same in the literature and are used interchangeably in the present application.

35 **[0031]** As used herein, the term "oligonucleotide specific for" or "oligonucleotide which targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of a mRNA transcript of the targeted gene. Stability of the complexes and duplexes can be determined by theoretical calculations and/or in vitro assays. Exemplary assays for determining stability of hybridization complexes and duplexes are described in the Examples below.

40 **[0032]** As used herein, the term "target nucleic acid" encompasses DNA, RNA (comprising premRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA, coding, noncoding sequences, sense or antisense polynucleotides. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds, which specifically hybridize to it, is generally referred to as "antisense". The functions of DNA to be interfered include, for example, replication and transcription. The functions of RNA to be interfered, include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of an encoded product or oligonucleotides.

45 **[0033]** RNA interference "RNAi" is mediated by double stranded RNA (dsRNA) molecules that have sequence-specific homology to their "target" nucleic acid sequences. In certain embodiments of the present invention, the mediators are 5-25 nucleotide "small interfering" RNA duplexes (siRNAs). The siRNAs are derived from the processing of dsRNA by an RNase enzyme known as Dicer. siRNA duplex products are recruited into a multi-protein siRNA complex termed RISC (RNA Induced Silencing Complex). Without wishing to be bound by any particular theory, a RISC is then believed to be guided to a target nucleic acid (suitably mRNA), where the siRNA duplex interacts in a sequence-specific way to mediate cleavage in a catalytic fashion. Small interfering RNAs that can be used in accordance with the present invention can be synthesized and used according to procedures that are well known in the art and that will be familiar to the ordinarily skilled artisan. Small interfering RNAs for use in the methods of the present invention suitably comprise between 50 about 1 to about 50 nucleotides (nt). In examples of non limiting embodiments, siRNAs can comprise about 5 to about 40 nt, about 5 to about 30 nt, about 10 to about 30 nt, about 15 to about 25 nt, or about 20-25 nucleotides.

55 **[0034]** Selection of appropriate oligonucleotides is facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Com-

parison of nucleic acid sequences from a range of species allows the selection of nucleic acid sequences that display an appropriate degree of identity between species. In the case of genes that have not been sequenced, Southern blots are performed to allow a determination of the degree of identity between genes in target species and other species. By performing Southern blots at varying degrees of stringency, as is well known in the art, it is possible to obtain an approximate measure of identity. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences in a subject to be controlled and a lower degree of complementarity to corresponding nucleic acid sequences in other species. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

**[0035]** By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, (1988) J. American. Med. Assoc. 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

**[0036]** By "decoy RNA" is meant an RNA molecule-that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

**[0037]** As used herein, the term "monomers" typically indicates monomers linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, e.g., from about 3-4, to about several hundreds of monomeric units. Analogs of phosphodiester linkages include: phosphorothioate, phosphorodithioate, methylphosphonates, phosphoroselenoate, phosphoramidate, and the like, as more fully described below.

**[0038]** The term "nucleotide" covers naturally occurring nucleotides as well as nonnaturally occurring nucleotides. It should be clear to the person skilled in the art that various nucleotides which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleotides" includes not only the known purine and pyrimidine heterocycles-containing molecules, but also heterocyclic analogues and tautomers thereof. Illustrative examples of other types of nucleotides are molecules containing adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo-N6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N4,N4-ethanocytosin, N6,N6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C3-C6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleotides described in Benner et al., U.S. Pat No. 5,432,272. The term "nucleotide" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleotides are those containing adenine, guanine, thymine, cytosine, and uracil, which are considered as the naturally occurring nucleotides in relation to therapeutic and diagnostic application in humans. Nucleotides include the natural 2'-deoxy and 2'-hydroxyl sugars, e.g., as described in Komberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992) as well as their analogs.

**[0039]** "Analog" in reference to nucleotides includes synthetic nucleotides having modified base moieties and/or modified sugar moieties (see e.g., described generally by Scheit, Nucleotide Analogs, John Wiley, New York, 1980; Freier & Altmann. (1997) Nucl. Acid. Res., 25(22). 4429-4443, Toulmé, J.J., (2001) Nature Biotechnology 19:17-18; Manoharan M., (1999) Biochemica et Biophysica Acta 1489:117-139, Freier S. M., (1997) Nucleic Acid Research, 25:4429-4443, Uhman, E., (2000) Drug Discovery & Development, 3: 203-213, Herdewin P., (2000) Antisense & Nucleic Acid Drug Dev., 10:297-310); 2'-O, 3'-C-linked [3.2.0] bicycloarabinonucleosides. Such analogs include synthetic nucleotides designed to enhance binding properties, e.g., duplex or triplex stability, specificity, or the like.

**[0040]** As used herein, "hybridization" means the pairing of substantially complementary strands of oligomeric compounds. One mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleotides) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleotides which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

**[0041]** An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

**[0042]** As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances and in the context of this invention, "stringent conditions" under which oligomeric compounds hybridize to a target sequence are determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated. In general,

stringent hybridization conditions comprise low concentrations (<0.15M) of salts with inorganic cations such as Na<sup>++</sup> or K<sup>++</sup> (i.e., low ionic strength), temperature higher than 20°C - 25° C. below the T<sub>m</sub> of the oligomeric compound:target sequence complex, and the presence of denaturants such as formamide, dimethylformamide, dimethyl sulfoxide, or the detergent sodium dodecyl sulfate (SDS). For example, the hybridization rate decreases 1.1% for each 1% formamide. An example of a high stringency hybridization condition is 0.1X sodium chloride-sodium citrate buffer (SSC)/0.1% (w/v) SDS at 60° C. for 30 minutes.

**[0043]** "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides on one or two oligomeric strands. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleotides such that stable and specific binding occurs between the oligomeric compound and a target nucleic acid.

**[0044]** It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure). The oligomeric compounds of the present invention comprise at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleotides of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides. As such, an antisense compound which is 18 nucleotides in length having 4 (four) non-complementary nucleotides which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art. Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., (1981) 2, 482-489).

**[0045]** As used herein, the term "Thermal Melting Point (T<sub>m</sub>)" refers to the temperature, under defined ionic strength, pH, and nucleic acid concentration, at which 50% of the oligonucleotides complementary to the target sequence hybridize to the target sequence at equilibrium. Typically, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short oligonucleotides (e.g., 10 to 50 nucleotide). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

**[0046]** As used herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene.

**[0047]** The term "variant", when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to a wild type gene. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. Of particular utility in the invention are variants of wild type gene products. Variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes that give rise to variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

**[0048]** The resulting polypeptides generally will, have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs,) or single base mutations in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population with a propensity for a disease state, that is susceptibility versus resistance.

**[0049]** Derivative polynucleotides include nucleic acids subjected to chemical modification, for example, replacement of hydrogen by an alkyl, acyl, or amino group. Derivatives, e.g., derivative oligonucleotides, may comprise non-naturally-occurring portions, such as altered sugar moieties or inter-sugar linkages. Exemplary among these are phosphorothioate and other sulfur containing species which are known in the art. Derivative nucleic acids may also contain labels, including

radionucleotides, enzymes, fluorescent agents, chemiluminescent agents, chromogenic agents, substrates, cofactors, inhibitors, magnetic particles, and the like.

**[0050]** A "derivative" polypeptide or peptide is one that is modified, for example, by glycosylation, pegylation, phosphorylation, sulfation, reduction/alkylation, acylation, chemical coupling, or mild formalin treatment. A derivative may also be modified to contain a detectable label, either directly or indirectly, including, but not limited to, a radioisotope, fluorescent, and enzyme label.

**[0051]** As used herein, the term "animal" or "patient" is meant to include, for example, humans, sheep, elks, deer, mule deer, minks, mammals, monkeys, horses, cattle, pigs, goats, dogs, cats, rats, mice, birds, chicken, reptiles, fish, insects and arachnids.

**[0052]** "Mammal" covers warm blooded mammals that are typically under medical care (e.g., humans and domesticated animals). Examples include feline, canine, equine, bovine, and human, as well as just human.

**[0053]** "Treating" or "treatment" covers the treatment of a disease-state in a mammal, and includes: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, e.g., arresting its development; and/or (c) relieving the disease-state, e.g., causing regression of the disease state until a desired endpoint is reached. Treating also includes the amelioration of a symptom of a disease (e.g., lessen the pain or discomfort), wherein such amelioration may or may not be directly affecting the disease (e.g., cause, transmission, expression, etc.).

**[0054]** As used herein, "cancer" refers to all types of cancer or neoplasm or malignant tumors found in mammals, including, but not limited to: leukemias, lymphomas, melanomas, carcinomas and sarcomas. The cancer manifests itself as a "tumor" or tissue comprising malignant cells of the cancer. Examples of tumors include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. Additional cancers which can be treated by the disclosed composition according to the invention include but not limited to, for example, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulinoma, malignant carcinoid, urinary bladder cancer, gastric cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, and prostate cancer.

**[0055]** As used herein, the term "vascular disease or disorder" refers to a disease of the circulatory system. Vascular diseases include arterial diseases, such as coronary artery disease (CAD), peripheral artery disease (PAD), abdominal aortic aneurysm; and venous diseases, such as blood clots, deep vein thrombosis, venous stasis disease, phlebitis, varicose veins. Atherosclerosis is the underlying cause of most vascular diseases, and thus a subject having atherosclerosis is a candidate for treatment with the compositions and methods described herein.

**[0056]** A cardiovascular disease or disorder includes those disorders that can either cause ischemia or are caused by reperfusion of the heart. Examples include but are not limited to, atherosclerosis, coronary artery disease, granulomatous myocarditis, chronic myocarditis (non-granulomatous), primary hypertrophic cardiomyopathy, peripheral artery disease (PAD), stroke, angina pectoris, myocardial infarction, cardiovascular tissue damage caused by cardiac arrest, cardiovascular tissue damage caused by cardiac bypass, cardiogenic shock, and related conditions that would be known by those of ordinary skill in the art or which involve dysfunction of or tissue damage to the heart or vasculature, especially, but not limited to, tissue damage related to ADAM activation. CVS diseases include, but are not limited to, atherosclerosis, granulomatous myocarditis, myocardial infarction, myocardial fibrosis secondary to valvular heart disease, myocardial fibrosis without infarction, primary hypertrophic cardiomyopathy, and chronic myocarditis (non-granulomatous). As used herein, "cardiomyopathy" refers to any disease or dysfunction of the myocardium (heart muscle) in which the heart is abnormally enlarged, thickened and/or stiffened. As a result, the heart muscle's ability to pump blood is usually weakened. The disease or disorder can be, for example, inflammatory, metabolic, toxic, infiltrative, fibroplastic, hematological, genetic, or unknown in origin. Such cardiomyopathies may result from a lack of oxygen. Other diseases include those

that result from myocardial injury which involves damage to the muscle or the myocardium in the wall of the heart as a result of disease or trauma. Myocardial injury can be attributed to many things such as, but not limited to, cardiomyopathy, myocardial infarction, or congenital heart disease. Specific cardiac disorders to be treated also include congestive heart failure, ventricular or atrial septal defect, congenital heart defect or ventricular aneurysm. The cardiac disorder may be

pediatric in origin. Cardiomyopathy includes but is not limited to, cardiomyopathy (dilated, hypertrophic, restrictive, arrhythmogenic and unclassified cardiomyopathy), acute and chronic heart failure, right heart failure, left heart failure, biventricular heart failure, congenital heart defects, mitral valve stenosis, mitral valve insufficiency, aortic valve stenosis, aortic valve insufficiency, tricuspidal valve stenosis, tricuspidal valve insufficiency, pulmonary valve stenosis, pulmonary valve insufficiency, combined valve defects, myocarditis, acute myocarditis, chronic myocarditis, viral myocarditis, diastolic heart failure, systolic heart failure, diabetic heart failure and accumulation diseases.

**[0057]** A "Metabolic disease or disorder" refers to a wide range of diseases and disorders of the endocrine system including, for example, insulin resistance, diabetes, obesity, impaired glucose tolerance, high blood cholesterol, hyperglycemia, hyperinsulinemia, dyslipidemia and hyperlipidemia.

#### *Polynucleotide and Oligonucleotide Compositions and Molecules*

**[0058]** *Targets:* In one embodiment, the targets comprise nucleic acid sequences of Fibroblast growth factor 21 (FGF21), including without limitation sense and/or antisense noncoding and/or coding sequences associated with FGF21.

**[0059]** FGF21 is a secreted polypeptide that belongs to a subfamily of fibroblast growth factors (FGFs) that includes FGF19, FGF21, and FGF23. FGF21 is an atypical FGF in that it is heparin independent and functions as a hormone in the regulation of glucose, lipid, and energy metabolism. FGF21 was isolated from a liver cDNA library as a hepatic secreted factor. It is highly expressed in liver and pancreas and is the only member of the FGF family to be primarily expressed in liver. Transgenic mice overexpressing FGF21 exhibit metabolic phenotypes of slow growth rate, low plasma glucose and triglyceride levels, and an absence of age-associated type 2 diabetes, islet hyperplasia, and obesity.

**[0060]** Fibroblast growth factor 21 Fibroblast growth factor 21 (FGF21) was identified based on cDNA sequence homology to other FGFs. Phylogenetic and structural analyses have assigned FGF21 to the FGF19 subfamily, which consists of FGF15 (the mouse ortholog of human FGF19), FGF19, FGF21, and FGF23. The FGF19 subfamily members distinguish themselves from the other 15 FGFs in that they function in an endocrine fashion. FGF23 is secreted primarily from bone and acts on the kidney to inhibit phosphate reabsorption and vitamin D biosynthesis. FGF15 is expressed by intestinal epithelium and is involved in the negative feedback regulation of bile acid synthesis in the liver. FGF21 is expressed predominantly in the liver and has emerged as a metabolic regulator of glucose uptake in adipocytes during a search for novel agents with therapeutic potential to treat diabetes mellitus. Administration of recombinant FGF21 lowered blood glucose levels in both obese mice and in diabetic mice. Furthermore, transgenic mice that overexpress FGF21 were hypoglycemic, sensitive to insulin, and resistant to diet-induced obesity.

**[0061]** In an embodiment, antisense oligonucleotides are used to prevent or treat diseases or disorders associated with FGF21 family members. Exemplary Fibroblast growth factor 21 (FGF21) mediated diseases and disorders which can be treated with cell/tissues regenerated from stem cells obtained using the antisense compounds comprise: a disease or disorder associated with abnormal function and/or expression of FGF21, a metabolic disease or disorder (e.g., diabetes, obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, hypertension, hepatosteatosis such as non-alcoholic steatohepatitis (NASH) etc.), cancer, a disease or disorder associated with impaired lipid metabolism a disease or disorder associated with impaired renal function, a disease or disorder associated with impaired hepatic function, abnormal cell proliferation, a vascular disease or disorder (e.g., coronary artery disease, peripheral artery disease, atherosclerosis, abdominal aortic aneurysm, a blood clot, deep vein thrombosis, venous stasis disease, phlebitis, varicose veins etc.), angiogenesis, atherosclerosis, a cardiovascular disease or disorder, a disease or disorder associated with impaired blood vessel formation, a disease or disorder associated with impaired cell signaling, a disease or disorder associated with impaired kinase activity, a disease or disorder associated with impaired uptake of glucose into adipocytes, a disease or disorder associated with impaired interactions between FGF21 and one or both of FGFR-1 or FGFR-2, a disease or disorder associated with impaired phosphorylation of FGFR-1 or FGFR-2 protein, and apoptosis.

**[0062]** In an embodiment, modulation of FGF21 by one or more antisense oligonucleotides is administered to a patient in need thereof, to prevent or treat any disease or disorder related to FGF21 abnormal expression, function, activity as compared to a normal control.

**[0063]** In an embodiment, the oligonucleotides are specific for polynucleotides of FGF21, which includes, without limitation noncoding regions. The FGF21 targets comprise variants of FGF21; mutants of FGF21, including SNPs; noncoding sequences of FGF21; alleles, fragments and the like. Preferably the oligonucleotide is an antisense RNA molecule.

**[0064]** In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to FGF21 polynucleotides alone but extends to any of the isoforms, receptors, homologs, non-coding regions and the like of FGF21.

**[0065]** In an embodiment, an oligonucleotide targets a natural antisense sequence (natural antisense to the coding

and non-coding regions) of FGF21 targets, including, without limitation, variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense RNA or DNA molecule.

5 [0066] In an embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenine, variants may be produced which contain thymidine, guanosine, cytidine or other natural or unnatural nucleotides at this position. This may be done at any of the positions of the antisense compound. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

10 [0067] In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

15 [0068] An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired. Such conditions include, i.e., physiological conditions in the case of in vivo assays or therapeutic treatment, and conditions in which assays are performed in the case of in vitro assays.

20 [0069] An antisense compound, whether DNA, RNA, chimeric, substituted etc, is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed.

25 [0070] In an embodiment, targeting of FGF21 including without limitation, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc., one or more of the sequences set forth as SEQ ID NOS: 2, and the like, modulate the expression or function of FGF21. In one embodiment, expression or function is up-regulated as compared to a control. In an embodiment, expression or function is down-regulated as compared to a control.

30 [0071] In an embodiment, oligonucleotides comprise nucleic acid sequences set forth as SEQ ID NOS: 3 to 9 including antisense sequences which are identified and expanded, using for example, PCR, hybridization etc. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the like. In an embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

35 [0072] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans.

40 [0073] In embodiments of the present invention oligomeric antisense compounds, particularly oligonucleotides, bind to target nucleic acid molecules and modulate the expression and/or function of molecules encoded by a target gene. The functions of DNA to be interfered comprise, for example, replication and transcription. The functions of RNA to be interfered comprise all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The functions may be up-regulated or inhibited depending on the functions desired.

45 [0074] The antisense compounds, include, antisense oligomeric compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds that hybridize to at least a portion of the target nucleic acid. As such, these compounds may be introduced in the form of single-stranded, double-stranded, partially single-stranded, or circular oligomeric compounds.

50 [0075] Targeting an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes Fibroblast growth factor 21 (FGF21).

**[0076]** The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

**[0077]** In an embodiment, the antisense oligonucleotides bind to the natural antisense sequences of Fibroblast growth factor 21 (FGF21) and modulate the expression and/or function of FGF21 (SEQ ID NO: 1). Examples of antisense sequences include SEQ ID NOS: 2 to 9.

**[0078]** In an embodiment, the antisense oligonucleotides bind to one or more segments of Fibroblast growth factor 21 (FGF21) polynucleotides and modulate the expression and/or function of FGF21. The segments comprise at least five consecutive nucleotides of the FGF21 sense or antisense polynucleotides.

**[0079]** In an embodiment, the antisense oligonucleotides are specific for natural antisense sequences of FGF21 wherein binding of the oligonucleotides to the natural antisense sequences of FGF21 modulate expression and/or function of FGF21.

**[0080]** In an embodiment, oligonucleotide compounds comprise sequences set forth as SEQ ID NOS: 3 to 9, antisense sequences which are identified and expanded, using for example, PCR, hybridization etc. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like. Examples of modified bonds or internucleotide linkages comprise phosphorothioate, phosphorodithioate or the like. In an embodiment, the nucleotides comprise a phosphorus derivative. The phosphorus derivative (or modified phosphate group) which may be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention may be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphorothioate and the like. The preparation of the above-noted phosphate analogs, and their incorporation into nucleotides, modified nucleotides and oligonucleotides, per se, is also known and need not be described here.

**[0081]** Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes has a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG; and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). Eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding Fibroblast growth factor 21 (FGF21), regardless of the sequence(s) of such codons. A translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

**[0082]** The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the "start codon region" (or "translation initiation codon region") and the "stop codon region" (or "translation termination codon region") are all regions that may be targeted effectively with the antisense compounds of the present invention.

**[0083]** The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Within the context of the present invention, a targeted region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

**[0084]** Another target region includes the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene). Still another target region includes the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. Another target region for this invention is the 5' cap region.

**[0085]** Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions

arc known as "exons" and arc spliced together to form a continuous mRNA sequence. In one embodiment, targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, is particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. An aberrant fusion junction due to rearrangement or deletion is another embodiment of a target site. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources arc known as "fusion transcripts". Introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

**[0086]** In an embodiment, the antisense oligonucleotides bind to coding and/or non-coding regions of a target polynucleotide and modulate the expression and/or function of the target molecule.

**[0087]** In an embodiment, the antisense oligonucleotides bind to natural antisense polynucleotides and modulate the expression and/or function of the target molecule.

**[0088]** In an embodiment, the antisense oligonucleotides bind to sense polynucleotides and modulate the expression and/or function of the target molecule.

**[0089]** Alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

**[0090]** Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

**[0091]** Variants can be produced through the use of alternative signals to start or stop transcription. Pre-mRNAs and mRNAs can possess more than one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also embodiments of target nucleic acids.

**[0092]** The locations on the target nucleic acid to which the antisense compounds hybridize are defined as at least a 5-nucleotide long portion of a target region to which an active antisense compound is targeted.

**[0093]** While the specific sequences of certain exemplary target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional target segments are readily identifiable by one having ordinary skill in the art in view of this disclosure.

**[0094]** Target segments 5-100 nucleotides in length comprising a stretch of at least five (5) consecutive nucleotides selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

**[0095]** Target segments can include DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 5'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). Similarly preferred target segments are represented by DNA or RNA sequences that comprise at least the 5 consecutive nucleotides from the 3'-terminus of one of the illustrative preferred target segments (the remaining nucleotides being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 5 to about 100 nucleotides). One having skill in the art armed with the target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

**[0096]** Once one or more target regions, segments or sites have been identified, antisense compounds arc chosen which arc sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

**[0097]** In embodiments of the invention the oligonucleotides bind to an antisense strand of a particular target. The oligonucleotides are at least 5 nucleotides in length and can be synthesized so each oligonucleotide targets overlapping sequences such that oligonucleotides are synthesized to cover the entire length of the target polynucleotide. The targets also include coding as well as non coding regions.

**[0098]** In one embodiment, it is preferred to target specific nucleic acids by antisense oligonucleotides. Targeting an antisense compound to a particular nucleic acid is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a non coding polynucleotide such as for example, non coding RNA (ncRNA).

**[0099]** RNAs can be classified into (1) messenger RNAs (mRNAs), which arc translated into proteins, and (2) non-

protein-coding RNAs (ncRNAs). ncRNAs comprise microRNAs, antisense transcripts and other Transcriptional Units (TU) containing a high density of stop codons and lacking any extensive "Open Reading Frame". Many ncRNAs appear to start from initiation sites in 3' untranslated regions (3'UTRs) of protein-coding loci. ncRNAs are often rare and at least half of the ncRNAs that have been sequenced by the FANTOM consortium seem not to be polyadenylated. Most researchers have for obvious reasons focused on polyadenylated mRNAs that are processed and exported to the cytoplasm. Recently, it was shown that the set of non-polyadenylated nuclear RNAs may be very large, and that many such transcripts arise from so-called intergenic regions. The mechanism by which ncRNAs may regulate gene expression is by base pairing with target transcripts. The RNAs that function by base pairing can be grouped into (1) cis encoded RNAs that are encoded at the same genetic location, but on the opposite strand to the RNAs they act upon and therefore display perfect complementarity to their target, and (2) trans-encoded RNAs that are encoded at a chromosomal location distinct from the RNAs they act upon and generally do not exhibit perfect base-pairing potential with their targets.

**[0100]** Without wishing to be bound by theory, perturbation of an antisense polynucleotide by the antisense oligonucleotides described herein can alter the expression of the corresponding sense messenger RNAs. However, this regulation can either be discordant (antisense knockdown results in messenger RNA elevation) or concordant (antisense knockdown results in concomitant messenger RNA reduction). In these cases, antisense oligonucleotides can be targeted to overlapping or non-overlapping parts of the antisense transcript resulting in its knockdown or sequestration. Coding as well as non-coding antisense can be targeted in an identical manner and that either category is capable of regulating the corresponding sense transcripts - either in a concordant or discordant manner. The strategies that are employed in identifying new oligonucleotides for use against a target can be based on the knockdown of antisense RNA transcripts by antisense oligonucleotides or any other means of modulating the desired target

*Strategy 1:* In the case of discordant regulation, knocking down the antisense transcript elevates the expression of the conventional (sense) gene. Should that latter gene encode for a known or putative drug target, then knockdown of its antisense counterpart could conceivably mimic the action of a receptor agonist or an enzyme stimulant.

*Strategy 2:* In the case of concordant regulation, one could concomitantly knock down both antisense and sense transcripts and thereby achieve synergistic reduction of the conventional (sense) gene expression. If, for example, an antisense oligonucleotide is used to achieve knockdown, then this strategy can be used to apply one antisense oligonucleotide targeted to the sense transcript and another antisense oligonucleotide to the corresponding antisense transcript, or a single energetically symmetric antisense oligonucleotide that simultaneously targets overlapping sense and antisense transcripts.

**[0101]** According to the present invention, antisense compounds include antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, doublestranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines, however, in some embodiments, the gene expression or function is up regulated. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

**[0102]** Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-

like structure are "DNA-like" and those which have A-formlike structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

**[0103]** In an embodiment, the desired oligonucleotides or antisense compounds, comprise at least one of: antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

**[0104]** dsRNA can also activate gene expression, a mechanism that has been termed "small RNA-induced gene activation" or RNAa. dsRNAs targeting gene promoters induce potent transcriptional activation of associated genes. RNAa was demonstrated in human cells using synthetic dsRNAs, termed "small activating RNAs" (saRNAs). It is currently not known whether RNAa is conserved in other organisms.

**[0105]** Small double-stranded RNA (dsRNA), such as small interfering RNA (siRNA) and microRNA (miRNA), have been found to be the trigger of an evolutionary conserved mechanism known as RNA interference (RNAi). RNAi invariably leads to gene silencing via remodeling chromatin to thereby suppress transcription, degrading complementary mRNA, or blocking protein translation. However, in instances described in detail in the examples section which follows, oligonucleotides are shown to increase the expression and/or function of the Fibroblast growth factor 21 (FGF21) polynucleotides and encoded products thereof. dsRNAs may also act as small activating RNAs (saRNA). Without wishing to be bound by theory, by targeting sequences in gene promoters, saRNAs would induce target gene expression in a phenomenon referred to as dsRNA-induced transcriptional activation (RNAa).

**[0106]** In a further embodiment, the "preferred target segments" identified herein may be employed in a screen for additional compounds that modulate the expression of Fibroblast growth factor 21 (FGF21) polynucleotides. "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding FGF21 and which comprise at least a 5-nucleotide portion that is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding sense or natural antisense polynucleotides of FGF21 with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding FGF21 polynucleotides, e.g. SEQ ID NOS: 3 to 9. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding FGF21 polynucleotides, the modulator may then be employed in further investigative studies of the function of FGF21 polynucleotides, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

**[0107]** Targeting the natural antisense sequence preferably modulates the function of the target gene. For example, the FGF21 gene (e.g. accession number NM\_019113). In an embodiment, the target is an antisense polynucleotide of the FGF21 gene. In an embodiment, an antisense oligonucleotide targets sense and/or natural antisense sequences of FGF21 polynucleotides (e.g. accession number NM\_019113), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule and the targets include coding and noncoding regions of antisense and/or sense FGF21 polynucleotides.

**[0108]** The preferred target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

**[0109]** Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications. For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target

**[0110]** In an embodiment, an antisense oligonucleotide targets Fibroblast growth factor 21 (FGF21) polynucleotides (e.g. accession number NM\_019113), variants, alleles, isoforms, homologs, mutants, derivatives, fragments and complementary sequences thereto. Preferably the oligonucleotide is an antisense molecule.

**[0111]** In accordance with embodiments of the invention, the target nucleic acid molecule is not limited to FGF21 alone but extends to any of the isoforms, receptors, homologs and the like of FGF21 molecules.

**[0112]** In an embodiment, an oligonucleotide targets a natural antisense sequence of FGF21 polynucleotides, for example, polynucleotides set forth as SEQ ID NOS: 2, and any variants, alleles, homologs, mutants, derivatives, fragments and complementary sequences thereto. Examples of antisense oligonucleotides are set forth as SEQ ID NOS: 3 to 9.

**[0113]** In one embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of FGF21 antisense, including without limitation noncoding sense and/or antisense sequences associated with FGF21 polynucleotides and modulate expression and/or function of FGF21 molecules.

**[0114]** In an embodiment, the oligonucleotides are complementary to or bind to nucleic acid sequences of FGF21 natural antisense, set forth as SEQ ID NOS: 2 and modulate expression and/or function of FGF21 molecules.

**[0115]** In an embodiment, oligonucleotides comprise sequences of at least 5 consecutive nucleotides of SEQ ID NOS:

3 to 9 and modulate expression and/or function of FGF21 molecules.

**[0116]** The polynucleotide targets comprise FGF21, including family members thereof, variants of FGF21; mutants of FGF21, including SNPs; noncoding sequences of FGF21; alleles of FGF21; species variants, fragments and the like. Preferably the oligonucleotide is an antisense molecule.

**[0117]** In an embodiment, the oligonucleotide targeting FGF21 polynucleotides, comprise: antisense RNA, interference RNA (RNAi), short interfering RNA (siRNA); micro interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); or, small activating RNA (saRNA).

**[0118]** In an embodiment, targeting of Fibroblast growth factor 21 (FGF21) polynucleotides, e.g., SEQ ID NOS: 2 modulate the expression or function of these targets. In one embodiment, expression or function is up-regulated as compared to a control. In an embodiment, expression or function is down-regulated as compared to a control.

**[0119]** In an embodiment, antisense compounds comprise sequences set forth as SEQ ID NOS: 3 to 9. These oligonucleotides can comprise one or more modified nucleotides, shorter or longer fragments, modified bonds and the like.

**[0120]** In an embodiment, SEQ ID NOS: 3 to 9 comprise one or more LNA nucleotides. Table I shows exemplary antisense oligonucleotides useful in the methods of the invention.

**Table 1:**

Sequence ID	Antisense Sequence Name	Sequence
SEQ ID NO:3	CUR-1469	C*C*C*T*T*C*T*C*T*C*C*A*A*C*T*C*T*C*C*C
SEQ ID NO:4	CUR-1470	C*A*G*A*T*C*C*A*C*A*G*T*C*C*G*C*T*T*T
SEQ ID NO:5	CUR-1471	G*A*G*C*G*C*C*C*A*G*T*T*G*C*A*A*T*G*C*T
SEQ ID NO:6	CUR-1472	T*C*T*T*C*C*G*G*T*G*C*C*A*G*G*G*C*T*T*A
SEQ ID NO:7	CUR-1473	C*C*T*G*T*G*C*C*T*G*T*A*G*T*C*C*C*A*T*C*T
SEQ ID NO:8	CUR-1474	A*G*G*C*T*A*C*C*T*G*G*C*T*T*C*T*G*A*G*C
SEQ ID NO:9	CUR-1475	T*A*C*C*T*C*C*T*G*C*T*C*T*C*T*C*A*C*C*C

**[0121]** The modulation of a desired target nucleic acid can be carried out in several ways known in the art. For example, antisense oligonucleotides, siRNA etc. Enzymatic nucleic acid molecules (e.g., ribozymes) are nucleic acid molecules capable of catalyzing one or more of a variety of reactions, including the ability to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be used, for example, to target virtually any RNA transcript.

**[0122]** Because of their sequence-specificity, trans-cleaving enzymatic nucleic acid molecules show promise as therapeutic agents for human disease. Enzymatic nucleic acid molecules can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

**[0123]** In general, enzymatic nucleic acids with RNA cleaving activity act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

**[0124]** Several approaches such as in vitro selection (evolution) strategies (Orgel, (1979) Proc. R. Soc. London, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as cleavage and ligation of phosphodiester linkages and amide linkages.

**[0125]** The development of ribozymes that are optimal for catalytic activity would contribute significantly to any strategy that employs RNA-cleaving ribozymes for the purpose of regulating gene expression. The hammerhead ribozyme, for example, functions with a catalytic rate (k<sub>cat</sub>) of about 1 min<sup>-1</sup> in the presence of saturating (10 mM) concentrations of Mg<sup>2+</sup> cofactor. An artificial "RNA ligase" ribozyme has been shown to catalyze the corresponding self-modification reaction with a rate of about 100 min<sup>-1</sup>. In addition, it is known that certain modified hammerhead ribozymes that have substrate binding arms made of DNA catalyze RNA cleavage with multiple turn-over rates that approach 100 min<sup>-1</sup>. Finally, replacement of a specific residue within the catalytic core of the hammerhead with certain nucleotide analogues gives modified ribozymes that show as much as a 10-fold improvement in catalytic rate. These findings demonstrate that ribozymes can promote chemical transformations with catalytic rates that are significantly greater than those dis-

played in vitro by most natural self-cleaving ribozymes. It is then possible that the structures of certain self-cleaving ribozymes may be optimized to give maximal catalytic activity, or that entirely new RNA motifs can be made that display significantly faster rates for RNA phosphodiester cleavage.

5 [0126] Intermolecular cleavage of an RNA substrate by an RNA catalyst that fits the "hammerhead" model was first shown in 1987 (Uhlenbeck, O. C. (1987) Nature, 328: 596-600). The RNA catalyst was recovered and reacted with multiple RNA molecules, demonstrating that it was truly catalytic.

[0127] Catalytic RNAs designed based on the "hammerhead" motif have been used to cleave specific target sequences by making appropriate base changes in the catalytic RNA to maintain necessary base pairing with the target sequences. This has allowed use of the catalytic RNA to cleave specific target sequences and indicates that catalytic RNAs designed according to the "hammerhead" model may possibly cleave specific substrate RNAs in vivo.

10 [0128] RNA interference (RNAi) has become a powerful tool for modularizing gene expression in mammals and mammalian cells. This approach requires the delivery of small interfering RNA (siRNA) either as RNA itself or as DNA, using an expression plasmid or virus and the coding sequence for small hairpin RNAs that are processed to siRNAs. This system enables efficient transport of the pre-siRNAs to the cytoplasm where they are active and permit the use of regulated and tissue specific promoters for gene expression.

15 [0129] In an embodiment, an oligonucleotide or antisense compound comprises an oligomer or polymer of ribonucleic acid (RNA) and/or deoxyribonucleic acid (DNA), or a mimetic, chimera, analog or homolog thereof. This term includes oligonucleotides composed of naturally occurring nucleotides, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often desired over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

20 [0130] According to the present invention, the oligonucleotides or "antisense compounds" include antisense oligonucleotides (e.g. RNA, DNA, mimetic, chimera, analog or homolog thereof), ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, saRNA, aRNA, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid and modulate its function. As such, they may be DNA, RNA, DNA-like, RNA-like, or mixtures thereof, or may be mimetics of one or more of these. These compounds may be single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. Antisense compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and/or branched. Antisense compounds can include constructs such as, for example, two strands hybridized to form a wholly or partially double-stranded compound or a single strand with sufficient self-complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. The two strands can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single stranded character. The double stranded compounds optionally can include overhangs on the ends. Further modifications can include conjugate groups attached to one of the termini, selected nucleotide positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex. Thus, the dsRNAs can be fully or partially double stranded. Specific modulation of gene expression can be achieved by stable expression of dsRNA hairpins in transgenic cell lines. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

25 [0131] Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect cleavage or other modification of the target nucleic acid or may work via occupancy-based mechanisms. In general, nucleic acids (including oligonucleotides) may be described as "DNA-like" (i.e., generally having one or more 2'-deoxy sugars and, generally, T rather than U bases) or "RNA-like" (i.e., generally having one or more 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Nucleic acid helices can adopt more than one type of structure, most commonly the A- and B-forms. It is believed that, in general, oligonucleotides which have B-form-like structure are "DNA-like" and those which have A-form-like structure are "RNA-like." In some (chimeric) embodiments, an antisense compound may contain both A- and B-form regions.

30 [0132] The antisense compounds in accordance with this invention can comprise an antisense portion from about 5 to about 80 nucleotides (i.e. from about 5 to about 80 linked nucleosides) in length. This refers to the length of the antisense strand or portion of the antisense compound. In other words, a single-stranded antisense compound of the invention comprises from 5 to about 80 nucleotides, and a double-stranded antisense compound of the invention (such as a dsRNA, for example) comprises a sense and an antisense strand or portion of 5 to about 80 nucleotides in length. One of ordinary skill in the art will appreciate that this comprehends antisense portions of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76,

77, 78, 79, or 80 nucleotides in length, or any range therewithin.

**[0133]** In one embodiment, the antisense compounds of the invention have antisense portions of 10 to 50 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies oligonucleotides having antisense portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin. In some embodiments, the oligonucleotides are 15 nucleotides in length.

**[0134]** In one embodiment, the antisense or oligonucleotide compounds of the invention have antisense portions of 12 or 13 to 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies antisense compounds having antisense portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length, or any range therewithin.

**[0135]** In an embodiment, the oligomeric compounds of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the compound. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the antisense or dsRNA compounds. These compounds are then tested using the methods described herein to determine their ability to inhibit expression of a target nucleic acid.

**[0136]** In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is from about 40% to about 60%. In some embodiments, homology, sequence identity or complementarity, is from about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is from about 70% to about 80%. In some embodiments, homology, sequence identity or complementarity, is from about 80% to about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

**[0137]** In an embodiment, the antisense oligonucleotides, such as for example, nucleic acid molecules set forth in SEQ ID NOS: 2 to 9 comprise one or more substitutions or modifications. In one embodiment, the nucleotides are substituted with locked nucleic acids (LNA).

**[0138]** In an embodiment, the oligonucleotides target one or more regions of the nucleic acid molecules sense and/or antisense of coding and/or non-coding sequences associated with FGF21 and the sequences set forth as SEQ ID NOS: 1 and 2. The oligonucleotides are also targeted to overlapping regions of SEQ ID NOS: 1 and 2.

**[0139]** Certain preferred oligonucleotides of this invention are chimeric oligonucleotides. "Chimeric oligonucleotides" or "chimeras," in the context of this invention, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of antisense modulation of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. In one embodiment, a chimeric oligonucleotide comprises at least one region modified to increase target binding affinity, and, usually, a region that acts as a substrate for RNase H. Affinity of an oligonucleotide for its target (in this case, a nucleic acid encoding ras) is routinely determined by measuring the T<sub>m</sub> of an oligonucleotide/target pair, which is the temperature at which the oligonucleotide and target dissociate: dissociation is detected spectrophotometrically. The higher the T<sub>m</sub>, the greater is the affinity of the oligonucleotide for the target.

**[0140]** Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotides mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

**[0141]** In an embodiment, the region of the oligonucleotide which is modified comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In another embodiment, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher T<sub>m</sub> (i.e., higher target binding affinity) than; 2'-deoxyoligonucleotides against a given target. The effect of such increased affinity is to greatly enhance RNAi oligonucleotide inhibition of gene expression. RNase H is a cellular endonuclease that cleaves the RNA strand of RNA:DNA duplexes; activation of this enzyme therefore results in cleavage of the RNA target, and thus can greatly enhance the efficiency of RNAi inhibition. Cleavage of the RNA target can be routinely demonstrated by gel electrophoresis. In another

embodiment, the chimeric oligonucleotide is also modified to enhance nuclease resistance. Cells contain a variety of exo- and endo-nucleases which can degrade nucleic acids. A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide. Nuclease resistance is routinely measured by incubating oligonucleotides with cellular extracts or isolated nuclease solutions and measuring the extent of intact oligonucleotide remaining over time, usually by gel electrophoresis. Oligonucleotides which have been modified to enhance their nuclease resistance survive intact for a longer time than unmodified oligonucleotides. A variety of oligonucleotide modifications have been demonstrated to enhance or confer nuclease resistance. Oligonucleotides which contain at least one phosphorothioate modification are presently more preferred. In some cases, oligonucleotide modifications which enhance target binding affinity are also, independently, able to enhance nuclease resistance.

**[0142]** Specific examples of some preferred oligonucleotides envisioned for this invention include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly CH<sub>2</sub>-NH-O-CH<sub>2</sub>, CH<sub>2</sub>-N(CH<sub>3</sub>)-O-CH<sub>2</sub> [known as a methylene(methylimino) or MM1 backbone], CH<sub>2</sub>-O-N(CH<sub>3</sub>)-CH<sub>2</sub>, CH<sub>2</sub>-N(CH<sub>3</sub>)-N(CH<sub>3</sub>)-CH<sub>2</sub> and O-N(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>2</sub> backbones, wherein the native phosphodiester backbone is represented as O-P-O-CH<sub>2</sub>. The amide backbones disclosed by Dc Mesmacker et al. (1995) Acc. Chem. Res. 28:366-374 arc also preferred. Also preferred arc oligonucleotides having morpholino backbone structures (Summerton and Weller, U.S. Pat. No. 5,034,506). In other an embodiment, such as the peptide nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone. Oligonucleotides may also comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH, SH, SCH<sub>3</sub>, F, OCN, OCH<sub>3</sub>OCH<sub>3</sub>, OCH<sub>3</sub>O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub> or O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> where n is from 1 to about 10; C<sub>1</sub> to C<sub>10</sub> lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; C<sub>1</sub>; Br, CN; CF<sub>3</sub>; OCF<sub>3</sub>; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH<sub>3</sub>; SO<sub>2</sub>CH<sub>3</sub>; ONO<sub>2</sub>; NO<sub>2</sub>; N<sub>3</sub>; NH<sub>2</sub>; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy[2'-O-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, also known as 2'-O-(2-methoxyethyl)]. Other preferred modifications include 2'-methoxy(2'-O-CH<sub>3</sub>), 2'-propoxy(2'-OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

**[0143]** Oligonucleotides may also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleotides include nucleotides found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, as well as synthetic nucleotides, e.g., 2-aminoadenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalkylamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 8-azaguanine, 7-deazaguanine, N<sup>6</sup>(6-aminohexyl)adenine and 2,6-diaminopurine. A "universal" base known in the art, e.g., inosine, may be included. 5-Mc-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. and are presently preferred base substitutions.

**[0144]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, e.g., dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid. Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides arc known in the art, for example, U.S. Pat. Nos. 5,138,045, 5,218,105 and 5,459,255.

**[0145]** It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide. The present invention also includes oligonucleotides which are chimeric oligonucleotides as hereinbefore defined.

**[0146]** In another embodiment, the nucleic acid molecule of the present invention is conjugated with another moiety including but not limited to abasic nucleotides, polyether, polyamine, polyamides, peptides, carbohydrates, lipid, or polyhydrocarbon compounds. Those skilled in the art will recognize that these molecules can be linked to one or more of any nucleotides comprising the nucleic acid molecule at several positions on the sugar, base or phosphate group.

**[0147]** The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including

Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of one of ordinary skill in the art. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling VA) to synthesize fluorescently labeled, biotinylated or other modified oligonucleotides such as cholesterol-modified oligonucleotides.

**[0148]** In accordance with the invention, use of modifications such as the use of LNA monomers to enhance the potency, specificity and duration of action and broaden the routes of administration of oligonucleotides comprised of current chemistries such as MOE, ANA, FANA, PS etc. This can be achieved by substituting some of the monomers in the current oligonucleotides by LNA monomers. The LNA modified oligonucleotide may have a size similar to the parent compound or may be larger or preferably smaller. It is preferred that such LNA-modified oligonucleotides contain less than about 70%, more preferably less than about 60%, most preferably less than about 50% LNA monomers and that their sizes are between about 5 and 25 nucleotides, more preferably between about 12 and 20 nucleotides.

**[0149]** Preferred modified oligonucleotide backbones comprise, but not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

**[0150]** Representative United States patents that teach the preparation of the above phosphorus containing linkages comprise, but are not limited to, US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

**[0151]** Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts.

**[0152]** Representative United States patents that teach the preparation of the above oligonucleosides comprise, but are not limited to, US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

**[0153]** In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds comprise, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen, et al. (1991) Science 254, 1497-1500.

**[0154]** In an embodiment of the invention the oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular -CH<sub>2</sub>-NH-O-CH<sub>2</sub>-, -CH<sub>2</sub>-N(CH<sub>3</sub>)-O-CH<sub>2</sub>- known as a methylene (methyleneimino) or MMI backbone, -CH<sub>2</sub>-O-N(CH<sub>3</sub>)-CH<sub>2</sub>-, -CH<sub>2</sub>N(CH<sub>3</sub>)-N(CH<sub>3</sub>)-CH<sub>2</sub>- and -O-N(CH<sub>3</sub>)-CH<sub>2</sub>-CH<sub>2</sub>- wherein the native phosphodiester backbone is represented as -O-P-O-CH<sub>2</sub>- of the above referenced US patent no. 5,489,677, and the amide backbones of the above referenced US patent no. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced US patent no. 5,034,506.

**[0155]** Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C to CO alkyl or C<sub>2</sub> to CO alkenyl and alkynyl. Particularly preferred are O(CH<sub>2</sub>)<sub>n</sub>OmCH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>OCH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub>, O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub>ONH<sub>2</sub>, and OCCH<sub>2</sub>nON(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>)<sub>2</sub> where n and m can be from 1 to about 10. Other preferred oligonucleotides comprise

one of the following at the 2' position: C to CO, (lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH3, OCN, Cl, Br, CN, CF3, OCF3, SOCH3, SO2CH3, ONO2, NO2, N3, NH2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification comprises 2'-methoxyethoxy (2'-O-CH2CH2OCH3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) i.e., an alkoxyalkoxy group. A further preferred modification comprises 2'-dimethylaminoethoxy, i.e., a O(CH2)2ON(CH3)2 group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH2-O-CH2-N(CH2)2.

**[0156]** Other preferred modifications comprise 2'-methoxy (2'-O CH3), 2'-aminopropoxy (2'-O CH2CH2CH2NH2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures comprise, but are not limited to, US patent nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference.

**[0157]** Oligonucleotides may also comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleotides comprise the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleotides comprise other synthetic and natural nucleotides such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo-uracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanin and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

**[0158]** Further, nucleotides comprise those disclosed in United States Patent No. 3,687,808, those disclosed in The Concise Encyclopedia of Polymer Science And Engineering', pages 858-859, Kroschwitz, J.I., cd. John Wiley & Sons, 1990, those disclosed by Englisch et al., 'Angewandte Chemie, International Edition', 1991, 30, page 613, and those disclosed by Sanghvi, Y.S., Chapter 15, 'Antisense Research and Applications', pages 289-302, Crooke, S.T. and Lebleu, B. ca., CRC Press, 1993. Certain of these nucleotides are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These comprise 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, comprising 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., cds, 'Antisense Research and Applications', CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

**[0159]** Representative United States patents that teach the preparation of the above noted modified nucleotides as well as other modified nucleotides comprise, but are not limited to, US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

**[0160]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates, which enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide.

**[0161]** Such moieties comprise but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxysterol moiety.

**[0162]** Representative United States patents that teach the preparation of such oligonucleotides conjugates comprise, but are not limited to, US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

**[0163]** *Drug discovery:* The compounds of the present invention can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the compounds and preferred target segments identified herein in drug discovery efforts to elucidate relationships that exist between Fibroblast growth factor 21 (FGF21) polynucleotides and a disease state, phenotype, or condition. These methods include detecting or modulating FGF21 polynucleotides comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of FGF21 polynucleotides and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

*Assessing Up-regulation or Inhibition of Gene Expression:*

**[0164]** Transfer of an exogenous nucleic acid into a host cell or organism can be assessed by directly detecting the presence of the nucleic acid in the cell or organism. Such detection can be achieved by several methods well known in the art. For example, the presence of the exogenous nucleic acid can be detected by Southern blot or by a polymerase chain reaction (PCR) technique using primers that specifically amplify nucleotide sequences associated with the nucleic acid. Expression of the exogenous nucleic acids can also be measured using conventional methods including gene expression analysis. For instance, mRNA produced from an exogenous nucleic acid can be detected and quantified using a Northern blot and reverse transcription PCR (RT-PCR).

**[0165]** Expression of RNA from the exogenous nucleic acid can also be detected by measuring an enzymatic activity or a reporter protein activity. For example, antisense modulatory activity can be measured indirectly as a decrease or increase in target nucleic acid expression as an indication that the exogenous nucleic acid is producing the effector RNA. Based on sequence conservation, primers can be designed and used to amplify coding regions of the target genes. Initially, the most highly expressed coding region from each gene can be used to build a model control gene, although any coding or non coding region can be used. Each control gene is assembled by inserting each coding region between a reporter coding region and its poly(A) signal. These plasmids would produce an mRNA with a reporter gene in the upstream portion of the gene and a potential RNAi target in the 3' non-coding region. The effectiveness of individual antisense oligonucleotides would be assayed by modulation of the reporter gene. Reporter genes useful in the methods of the present invention include acetohydroxyacid synthase (AHAS), alkaline phosphatase (AP), beta galactosidase (LacZ), beta glucuronidase (GUS), chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), horseradish peroxidase (HRP), luciferase (Luc), nopaline synthase (NOS), octopine synthase (OCS), and derivatives thereof. Multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin, methotrexate, phosphinothricin, puromycin, and tetracycline. Methods to determine modulation of a reporter gene are well known in the art, and include, but are not limited to, fluorometric methods (e.g. fluorescence spectroscopy, Fluorescence Activated Cell Sorting (FACS), fluorescence microscopy), antibiotic resistance determination.

**[0166]** FGF21 protein and mRNA expression can be assayed using methods known to those of skill in the art and described elsewhere herein. For example, immunoassays such as the ELISA can be used to measure protein levels. FGF21 ELISA assay kits are available commercially, e.g., from R&D Systems (Minneapolis, MN).

**[0167]** In embodiments, FGF21 expression (e.g., mRNA or protein) in a sample (e.g., cells or tissues in vivo or in vitro) treated using an antisense oligonucleotide of the invention is evaluated by comparison with FGF21 expression in a control sample. For example, expression of the protein or nucleic acid can be compared using methods known to those of skill in the art with that in a mock-treated or untreated sample. Alternatively, comparison with a sample treated with a control antisense oligonucleotide (e.g., one having an altered or different sequence) can be made depending on the information desired. In another embodiment, a difference in the expression of the FGF21 protein or nucleic acid in a treated vs. an untreated sample can be compared with the difference in expression of a different nucleic acid (including any standard deemed appropriate by the researcher, e.g., a housekeeping gene) in a treated sample vs. an untreated sample.

**[0168]** Observed differences can be expressed as desired, e.g., in the form of a ratio or fraction, for use in a comparison with control. In embodiments, the level of FGF21 mRNA or protein, in a sample treated with an antisense oligonucleotide of the present invention, is increased or decreased by about 1.25-fold to about 10-fold or more relative to an untreated sample or a sample treated with a control nucleic acid. In embodiments, the level of FGF21 mRNA or protein is increased or decreased by at least about 1.25-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, or at least about 10-fold or more.

*Kits, Research Reagents, Diagnostics, and Therapeutics*

5 [0169] The compounds of the present invention can be utilized for diagnostics, therapeutics, and prophylaxis, and as research reagents and components of kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

10 [0170] For use in kits and diagnostics and in various biological systems, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, are useful as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

[0171] As used herein the term "biological system" or "system" is defined as any organism, cell, cell culture or tissue that expresses, or is made competent to express products of the Fibroblast growth factor 21 (FGF21) genes. These include, but are not limited to, humans, transgenic animals, cells, cell cultures, tissues, xenografts, transplants and combinations thereof.

15 [0172] As one non limiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds that affect expression patterns.

20 [0173] Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays, SAGE (serial analysis of gene expression), READS (restriction enzyme amplification of digested cDNAs), TOGA (total gene expression analysis), protein arrays and proteomics, expressed sequence tag (EST) sequencing, subtractive RNA fingerprinting (SuRF), subtractive cloning, differential display (DD), comparative genomic hybridization, FISH (fluorescent in situ hybridization) techniques and mass spectrometry methods.

25 [0174] The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding Fibroblast growth factor 21 (FGF21). For example, oligonucleotides that hybridize with such efficiency and under such conditions as disclosed herein as to be effective FGF21 modulators are effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding FGF21 and in the amplification of said nucleic acid molecules for detection or for use in further studies of FGF21. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding FGF21 can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabeling of the oligonucleotide, or any other suitable detection means. Kits using such detection means for detecting the level of FGF21 in a sample may also be prepared.

30 [0175] The specificity and sensitivity of antisense are also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

35 [0176] For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of FGF21 polynucleotides is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of FGF21 modulator. The FGF21 modulators of the present invention effectively modulate the activity of the FGF21 or modulate the expression of the FGF21 protein. In one embodiment, the activity or expression of FGF21 in an animal is inhibited by about 10% as compared to a control. Preferably, the activity or expression of FGF21 in an animal is inhibited by about 30%. More preferably, the activity or expression of FGF21 in an animal is inhibited by 50% or more. Thus, the oligomeric compounds modulate expression of Fibroblast growth factor 21 (FGF21) mRNA by at least 10%, by at least 50%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to a control.

40 [0177] In one embodiment, the activity or expression of Fibroblast growth factor 21 (FGF21) and/or in an animal is increased by about 10% as compared to a control. Preferably, the activity or expression of FGF21 in an animal is increased by about 30%. More preferably, the activity or expression of FGF21 in an animal is increased by 50% or more. Thus, the oligomeric compounds modulate expression of FGF21 mRNA by at least 10%, by at least 50%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100% as compared to

a control.

**[0178]** For example, the reduction of the expression of Fibroblast growth factor 21 (FGF21) may be measured in serum, blood, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic acid molecule encoding FGF21 peptides and/or the FGF21 protein itself.

**[0179]** The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

### Conjugates

**[0180]** Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamins, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application No. PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, which are incorporated herein by reference. Conjugate moieties include, but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate, a polyamine or a polyethylene glycol chain, or Adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triodobenzoic acid, flufenamic acid, folic acid, a benzothiadiazide, chlorothiazide, a diazepam, indomethacin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

**[0181]** Representative United States patents that teach the preparation of such oligonucleotides conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

### Formulations

**[0182]** The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,165; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

**[0183]** Although, the antisense oligonucleotides do not need to be administered in the context of a vector in order to modulate a target expression and/or function, embodiments of the invention relates to expression vector constructs for the expression of antisense oligonucleotides, comprising promoters, hybrid promoter gene sequences and possess a strong constitutive promoter activity, or a promoter activity which can be induced in the desired case.

**[0184]** In an embodiment, invention practice involves administering at least one of the foregoing antisense oligonucleotides with a suitable nucleic acid delivery system. In one embodiment, that system includes a non-viral vector operably linked to the polynucleotide. Examples of such nonviral vectors include the oligonucleotide alone (e.g. any one or more

of SEQ ID NOS: 3 to 9) or in combination with a suitable protein, polysaccharide or lipid formulation.

**[0185]** Additionally suitable nucleic acid delivery systems include viral vector, typically sequence from at least one of an adenovirus, adenovirus-associated virus (AAV), helper-dependent adenovirus, retrovirus, or hemagglutinin virus of Japan-liposome (HVJ) complex. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide e.g., a cytomegalovirus (CMV) promoter.

**[0186]** Additionally preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. One preferred HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the env gene is from another virus. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector, Adenovirus Vectors and Adeno-associated Virus Vectors.

**[0187]** The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

**[0188]** The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[0189]** The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

**[0190]** For treating tissues in the central nervous system, administration can be made by, e.g., injection or infusion into the cerebrospinal fluid. Administration of antisense RNA into cerebrospinal fluid is described, e.g., in U.S. Pat. App. Pub. No. 2007/0117772, "Methods for slowing familial ALS disease progression," incorporated herein by reference in its entirety.

**[0191]** When it is intended that the antisense oligonucleotide of the present invention be administered to cells in the central nervous system, administration can be with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier. Injection can be made, e.g., in the entorhinal cortex or hippocampus. Delivery of neurotrophic factors by administration of an adenovirus vector to motor neurons in muscle tissue is described in, e.g., U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, e.g., the striatum, the thalamus, the hippocampus, or the substantia nigra, is known in the art and described, e.g., in U.S. Pat. No. 6,756,523, "Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain," incorporated herein by reference. Administration can be rapid as by injection or made over a period of time as by slow infusion or administration of slow release formulations.

**[0192]** The subject antisense oligonucleotides can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, the antisense oligonucleotide can be coupled to any substance, known in the art to promote penetration or transport across the blood-brain barrier, such as an antibody to the transferrin receptor, and administered by intravenous injection. The antisense compound can be linked with a viral vector, for example, that makes the antisense compound more effective and/or increases the transport of the antisense compound across the blood-brain barrier. Osmotic blood brain barrier disruption can also be accomplished by, e.g., infusion of sugars including, but not limited to, meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids including, but not limited to, glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, e.g., in U.S. Patent No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," 6,294,520, "Material for passage through the blood-brain barrier," and 6,936,589, "Parenteral delivery systems," all incorporated herein by reference in their entirety.

**[0193]** The subject antisense compounds may be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. For example, cationic lipids may be included in the formulation to facilitate oligonucleotide uptake. One such composition shown to facilitate uptake is LIPOFECTIN (available from GIBCO-BRL, Bethesda, MD).

**[0194]** Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

**[0195]** The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

**[0196]** The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

**[0197]** Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

**[0198]** Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1  $\mu\text{m}$  in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug that may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860.

**[0199]** Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes that are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

**[0200]** Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids. When incorporated into liposomes, these specialized lipids result in liposomes with enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860.

**[0201]** The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[0202]** In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating nonsurfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[0203]** One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

**[0204]** Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoyl-phosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. diolcoyltetramethylaminopropyl DOTAP and diolcoyl-phosphatidyl ethanolamine DOTMA),

**[0205]** For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860.

**[0206]** Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticles, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or min-

tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers, surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein by reference.

**[0207]** Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

**[0208]** Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents that function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bischloroethyl-nitrosurca, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurca, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycytosine, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vinorelbine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribavirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

**[0209]** In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. For example, the first target may be a particular antisense sequence of Fibroblast growth factor 21 (FGF21), and the second target may be a region from another nucleotide sequence. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same Fibroblast growth factor 21 (FGF21) nucleic acid target. Numerous examples of antisense compounds are illustrated herein and others may be selected from among suitable compounds known in the art. Two or more combined compounds may be used together or sequentially.

#### *Dosing:*

**[0210]** The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC50s found to be effective *in vitro* and *in vivo* animal models. In general, dosage is from 0.01  $\mu$ g to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01  $\mu$ g to 100 g per kg of body weight, once or more daily, to once every 20 years.

**[0211]** In embodiments, a patient is treated with a dosage of drug that is at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40,

at least about 45, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, or at least about 100 mg/kg body weight. Certain injected dosages of antisense oligonucleotides are described, e.g., in U.S. Pat. No. 7,563,884, "Antisense modulation of PTPIB expression," incorporated herein by reference in its entirety.

5 [0212] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. Numerous changes to the disclosed embodiments can be made in accordance with the disclosure herein without departing from the spirit or scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described embodiments.

10 [0213] All documents mentioned herein are incorporated herein by reference. All publications and patent documents cited in this application are incorporated by reference for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention. Embodiments of inventive compositions and methods are illustrated in the following examples.

## 15 EXAMPLES

[0214] The following non-limiting Examples serve to illustrate selected embodiments of the invention. It will be appreciated that variations in proportions and alternatives in elements of the components shown will be apparent to those skilled in the art and arc within the scope of embodiments of the present invention.

20 *Example 1: Design of antisense oligonucleotides specific for a nucleic acid molecule antisense to a Fibroblast growth factor 21 (FGF21) and/or a sense strand of FGF21 polynucleotide*

25 [0215] As indicated above the term "oligonucleotide specific for" or "oligonucleotide targets" refers to an oligonucleotide having a sequence (i) capable of forming a stable complex with a portion of the targeted gene, or (ii) capable of forming a stable duplex with a portion of an mRNA transcript of the targeted gene.

[0216] Selection of appropriate oligonucleotides is facilitated by using computer programs (e.g. IDT AntiSense Design, IDT OligoAnalyzer) that automatically identify in each given sequence subsequences of 19-25 nucleotides that will form hybrids with a target polynucleotide sequence with a desired melting temperature (usually 50-60°C) and will not form self-dimers or other complex secondary structures

30 [0217] Selection of appropriate oligonucleotides is further facilitated by using computer programs that automatically align nucleic acid sequences and indicate regions of identity or homology. Such programs are used to compare nucleic acid sequences obtained, for example, by searching databases such as GenBank or by sequencing PCR products. Comparison of nucleic acid sequences from a range of genes and intergenic regions of a given genome allows the selection of nucleic acid sequences that display an appropriate degree of specificity to the gene of interest. These procedures allow the selection of oligonucleotides that exhibit a high degree of complementarity to target nucleic acid sequences and a lower degree of complementarity to other nucleic acid sequences in a given genome. One skilled in the art will realize that there is considerable latitude in selecting appropriate regions of genes for use in the present invention.

35 [0218] An antisense compound is "specifically hybridizable" when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a modulation of function and/or activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

40 [0219] The hybridization properties of the oligonucleotides described herein can be determined by one or more *in vitro* assays as known in the art. For e.g., the properties of the oligonucleotides described herein can be obtained by determination of binding strength between the target natural antisense and a potential drug molecules using melting curve assay.

45 [0220] The binding strength between the target natural antisense and a potential drug molecule (Molecule) can be estimated using any of the established methods of measuring the strength of intermolecular interactions, for example, a melting curve assay.

50 [0221] Melting curve assay determines the temperature at which a rapid transition from double-stranded to single-stranded conformation occurs for the natural antisense/Molecule complex. This temperature is widely accepted as a reliable measure of the interaction strength between the two molecules.

55 [0222] A melting curve assay can be performed using a cDNA copy of the actual natural antisense RNA molecule or a synthetic DNA or RNA nucleotide corresponding to the binding site of the Molecule. Multiple kits containing all necessary reagents to perform this assay are available (e.g. Applied Biosystems Inc. MeltDoctor kit). These kits include a suitable buffer solution containing one of the double strand DNA (dsDNA) binding dyes (such as ABI HRM dyes, SYBR Green, SYTO, etc.). The properties of the dsDNA dyes arc such that they emit almost no fluorescence in free form, but are

highly fluorescent when bound to dsDNA.

**[0223]** To perform the assay the cDNA or a corresponding oligonucleotide arc mixed with Molecule in concentrations defined by the particular manufacturer's protocols. The mixture is heated to 95 °C to dissociate all pre-formed dsDNA complexes, then slowly cooled to room temperature or other lower temperature defined by the kit manufacturer to allow the DNA molecules to anneal. The newly formed complexes are then slowly heated to 95 °C with simultaneous continuous collection of data on the amount of fluorescence that is produced by the reaction. The fluorescence intensity is inversely proportional to the amounts of dsDNA present in the reaction. The data can be collected using a real time PCR instrument compatible with the kit (e.g.ABI's StepOne Plus Real Time PCR System or lightTyper instrument, Roche Diagnostics, Lcwcs, UK).

**[0224]** Melting peaks are constructed by plotting the negative derivative of fluorescence with respect to temperature (-d(Fluorescence)/dT) on the y-axis) against temperature (x-axis) using appropriate software (for example lightTyper (Roche) or SDS Dissociation Curve, ABI). The data is analyzed to identify the temperature of the rapid transition from dsDNA complex to single strand molecules. This temperature is called T<sub>m</sub> and is directly proportional to the strength of interaction between the two molecules. Typically, T<sub>m</sub> will exceed 40 °C.

*Example 2: Modulation of FGF21 polynucleotides- Treatment of MCF-7 cells with antisense oligonucleotides*

**[0225]** All antisense oligonucleotides used in Example 2 were designed as described in Example 1. The manufacturer (IDT Inc. of Coralville, IA) was instructed to manufacture the designed phosphothioate bond oligonucleotides and provided the designed phosphothioate analogs shown in Table 1. The asterisk designation between nucleotides indicates the presence of phosphothioate bond. The oligonucleotides required for the experiment in Example 2 can be synthesized using any appropriate state of the art method, for example the method used by IDT: on solid support, such as a 5 micron controlled pore glass bead (CPG), using phosphoramidite monomers (normal nucleotides with all active groups protected with protection groups, e.g. trityl group on sugar, benzoyl on A and C and N-2-isobutyryl on G). Protection groups prevent the unwanted reactions during oligonucleotide synthesis. Protection groups are removed at the end of the synthesis process. The initial nucleotide is linked to the solid support through the 3' carbon and the synthesis proceeds in the 3' to 5' direction. The addition of a new base to a growing oligonucleotide chain takes place in four steps: 1) the protection group is removed from the 5' oxygen of the immobilized nucleotide using trichloroacetic acid; 2) the immobilized and the next-in-sequence nucleotides are coupled together using tetrazole; the reaction proceeds through a tetrazolyl phosphoramidite intermediate; 3) the unreacted free nucleotides and reaction byproducts arc washed away and the unreacted immobilized oligonucleotides arc capped to prevent their participation in the next round of synthesis; capping is achieved by acetylating the free 5' hydroxyl using acetic anhydride and N-methyl imidazole; 4) to stabilize the bond between the nucleotides the phosphorus is oxidized using iodine and water, if a phosphodiester bond is to be produced, or Beaucage reagent (3H-1,2-benzodithiol-3-one-1,1-dioxide), if a phosphothioate bond is desired. By alternating the two oxidizing agents, a chimeric backbone can be constructed. The four step cycle described above is repeated for every nucleotide in the sequence. When the complete sequence is synthesized, the oligonucleotide is cleaved from the solid support and deprotected using ammonium hydroxide at high temperature. Protection groups are washed away by desalting and the remaining oligonucleotides arc lyophilized. To perform the experiment designed in Example 2, MCF-7 cells from ATCC (cat# HTB-22) were grown in growth media were grown in growth media (DMEM (Mediatech cat # MT 10-013-CV) +10% FBS (Mcdiatcch cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO<sub>2</sub>. One day before the experiment the cells were replated at the density of 1.5 × 10<sup>5</sup>/ml into 6 well plates and incubated at 37°C and 5% CO<sub>2</sub> overnight. On the day of the experiment the media in the 6 well plates was changed to fresh growth media. Oligonucleotides shipped by the manufacturer in lyophilized form were diluted to the concentration of 20 μM in deionized RNase/DNase-free water. Two μl of this solution was incubated with 400 μl of OptiMEM media (Gibco cat#31985-070) and 4 μl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min, then applied dropwise to one well of the 6 well plate with MCF-7 cells. Similar mixture including 2 μl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO<sub>2</sub> the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers' instructions. 600 ng of extracted RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR. The principle of the real time PCR procedure is based on the ability of a polymerase chain reaction (PCR) to specifically amplify a fragment of the DNA or cDNA molecule of interest and thus make it detectable and quantifiable. PCR reaction is catalyzed by an enzyme called DNA polymerase. DNA polymerases are enzymes that are able to build a complementary DNA strand on an existing DNA or cDNA template starting at the end of a strctch of doublestranded DNA which can be as short as 18-22 nucleotides. For practical purposes in a PCR reaction two short synthetic DNA fragments complementary to the target DNA or cDNA

sequence (called forward and reverse primers) are used to provide a starting point for DNA polymerase. The reaction is conducted in a buffer with appropriate pH and salt concentrations which also contains cofactors necessary for the DNA polymerase activity and the building blocks for the newly created complementary DNA chain (deoxynucleotide triphosphates or dNTPs). Each PCR cycle will double the number of copies of the fragment of interest using forward or reverse primer in alternating fashion to initiate the reaction. Thermostable DNA polymerases used in PCR reactions are active at temperatures up to 72°C and are able to withstand heating to 95°C without losing activity. Thermostable DNA polymerases were originally purified from thermostable microorganisms populating thermal vents. Eventually genetically engineered versions of these DNA polymerases with improved characteristics were developed, for example AmpliTaq and AmpliTaqGold enzymes (Applied Biosystems Inc.). As opposed to conventional PCR, real time PCR reactions are able to not only amplify but also simultaneously quantify a DNA or cDNA molecule of interest. Quantification in absolute number of copies or in relative numbers normalized to an investigator-specified DNA sample is possible. The key difference of real time PCR as compared to standard PCR reaction is that the amounts of amplified DNA are measured as the reaction progresses in real time. Common methods for detection of products in real-time PCR include intercalation dyes that fluoresce when associated with double-stranded DNA and fluorescent sequence-specific DNA probes. DNA probes are oligonucleotides complementary to the DNA of interest that are labeled with a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher attached at the 3'-end. Several different fluorophores (e.g. 6-carboxyfluorescein, FAM, or tetraethylrhodamine, TET) and quenchers (e.g. tetramethylrhodamine, TAMRA, or dihydrocyclopyrroloindole tripeptide minor groove binder, MGB) are commonly used. As long as both fluorophore and quencher are attached to one oligonucleotide molecule the fluorescence emitted by the fluorophore upon excitation is quenched by the quencher via FRET (fluorescence resonance energy transfer). The probes are designed to be complementary to the DNA region between the PCR primers used in the reaction. As the DNA polymerase extends the primer, due to its 5' to 3' exonuclease activity it also degrades the probe. Degradation of the probe separates the fluorophore from the quencher, thus allowing the emitted fluorescence to escape. Fluorescence released at each PCR cycle is directly proportional to the amount of DNA template present in the PCR. Due to limited amounts of probe and dNTPs present in the reaction and eventual loss of polymerase activity most PCR reactions reach a plateau stage when the number of copies does not change in successive cycles. This represents a significant limitation for using conventional PCR where all reactions are assessed after a set number of cycles for DNA quantification. Monitoring fluorescence intensity at each cycle allows comparing individual PCR reactions in their linear amplification, not plateau, stage thus avoiding this limitation. To ensure that the difference in quantities of the DNA of interest between the samples was not due to different amounts of input RNA/cDNA, it is usually necessary to normalize expression of a target gene to a control gene unaffected by the test conditions. Real time PCR reaction can be conducted using a real time PCR machine such as StepOne Plus Real Time PCR Machine (Applied Biosystems).

**[0226]** Real time PCR reaction used in Example 2 was conducted using AB1 Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay: Hs00173927\_ml by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min. 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using StepOne Plus Real Time PCR Machine (Applied Biosystems). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

**[0227]** *Results:* Real time PCR results show that the levels of the FGF21 mRNA in MCF-7 cells are significantly increased 48 h after treatment with the oligos designed to FGF21 antisense Hs.69747 (Fig 1).

*Example 3: Modulation of FGF21 polynucleotides-Treatment of HepG2 cells with antisense oligonucleotides*

**[0228]** All antisense oligonucleotides used in Example 3 were designed as described in Example 1. The manufacturer (IDT Inc. of Coralville, IA) was instructed to manufacture the designed phosphothioate bond oligonucleotides and provided the designed phosphothioate analogs shown in Table I. The asterisk designation between nucleotides indicates the presence of phosphothioate bond. The oligonucleotides required for the experiment in Example 3 can be synthesized using any appropriate state of the art method, for example the method used by IDT: on solid support, such as a 5 micron controlled pore glass bead (CPG), using phosphoramidite monomers (normal nucleotides with all active groups protected with protection groups, e.g. trityl group on sugar, benzoyl on A and C and N-2-isobutyryl on G). Protection groups prevent the unwanted reactions during oligonucleotide synthesis. Protection groups are removed at the end of the synthesis process. The initial nucleotide is linked to the solid support through the 3' carbon and the synthesis proceeds in the 3' to 5' direction. The addition of a new base to a growing oligonucleotide chain takes place in four steps: 1) the protection group is removed from the 5' oxygen of the immobilized nucleotide using trichloroacetic acid; 2) the immobilized and the next-in-sequence nucleotides are coupled together using tetrazole; the reaction proceeds through a tetrazolyl phosphoramidite intermediate; 3) the unreacted free nucleotides and reaction byproducts are washed away and the unreacted immobilized oligonucleotides are capped to prevent their participation in the next round of synthesis; capping is achieved by acetylating the free 5' hydroxyl using acetic anhydride and N-methyl imidazole; 4) to stabilize the bond between the

nucleotides the phosphorus is oxidized using iodine and water, if a phosphodiester bond is to be produced, or Beaucage reagent (3H-1,2-benzodithiol-3-one-1,1-dioxide), if a phosphothioate bond is desired. By alternating the two oxidizing agents, a chimeric backbone can be constructed. The four step cycle described above is repeated for every nucleotide in the sequence. When the complete sequence is synthesized, the oligonucleotide is cleaved from the solid support and deprotected using ammonium hydroxide at high temperature. Protection groups are washed away by desalting and the remaining oligonucleotides are lyophilized.

**[0229]** To perform the experiment designed in Example 3, HepG2 cells from ATCC (cat# HB-8065) were grown in growth media (MEM/EBSS (Hyclone cat #SH30024, or Mediatech cat # MT-10-010-CV) +10% FBS (Mediatech cat# MT35- 011-CV)+ penicillin/streptomycin (Mediatech cat# MT30-002-CI)) at 37°C and 5% CO<sub>2</sub>. One day before the experiment the cells were replated at the density of 0.5x10<sup>4</sup>/ml into 6 well plates and incubated at 37°C and 5% CO<sub>2</sub> overnight. On the day of the experiment the media in the 6 well plates was changed to fresh growth media.

**[0230]** Oligonucleotides shipped by the manufacturer in lyophilized form were diluted to the concentration of 20 μM in deionized RNase/DNase-free water. Two μl of this solution was incubated with 400 μl of OptiMEM media (Gibco cat#31985-070) and 4 μl of Lipofectamine 2000 (Invitrogen cat# 11668019) at room temperature for 20 min, then applied dropwise to one well of the 6 well plate with HepG2 cells. Similar mixture including 2 μl of water instead of the oligonucleotide solution was used for the mock-transfected controls. After 3-18 h of incubation at 37°C and 5% CO<sub>2</sub> the media was changed to fresh growth media. 48 h after addition of antisense oligonucleotides the media was removed and RNA was extracted from the cells using SV Total RNA Isolation System from Promega (cat # Z3105) or RNeasy Total RNA Isolation kit from Qiagen (cat# 74181) following the manufacturers instructions. 600 ng of extracted RNA was added to the reverse transcription reaction performed using Verso cDNA kit from Thermo Scientific (cat#AB1453B) or High Capacity cDNA Reverse Transcription Kit (cat# 4368813) as described in the manufacturer's protocol. The cDNA from this reverse transcription reaction was used to monitor gene expression by real time PCR using ABI Taqman Gene Expression Mix (cat#4369510) and primers/probes designed by ABI (Applied Biosystems Taqman Gene Expression Assay: Hs00173927\_ml by Applied Biosystems Inc., Foster City CA). The following PCR cycle was used: 50°C for 2 min, 95°C for 10 min, 40 cycles of (95°C for 15 seconds, 60°C for 1 min) using StepOne Plus Real Time PCR Machine (Applied Biosystems). Fold change in gene expression after treatment with antisense oligonucleotides was calculated based on the difference in 18S-normalized dCt values between treated and mock-transfected samples.

**[0231] Results:** Real time PCR results show that the levels of the FGF21 mRNA in HepG2 cells are significantly increased 48 h after treatment with one of the oligos designed to FGF21 antisense Hs.69747 (Fig 2).

**[0232]** Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations and modifications will occur to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

**[0233]** The Abstract of the disclosure will allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the following claims.

**[0234]** Preferred embodiments of the present invention are described below and referred to as embodiments E1 to E37.

E1. A method of modulating a function of and/or the expression of a Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising:

contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a polynucleotide comprising 5 to 30 consecutive nucleotides within nucleotides 1 to 4246 of SEQ ID NO: 2; thereby modulating a function of and/or the expression of the Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

E2. A method of modulating a function of and/or the expression of a Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising:

contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said at least one oligonucleotide has at least 50% sequence identity to a reverse complement of a natural antisense of a Fibroblast growth factor 21 (FGF21) polynucleotide; thereby modulating a function of and/or the expression of the Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

E3. A method of modulating a function of and/or the expression of a Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro* comprising:

contacting said cells or tissues with at least one antisense oligonucleotide 5 to 30 nucleotides in length wherein said oligonucleotide has at least 50% sequence identity to an antisense oligonucleotide to the Fibroblast growth factor 21 (FGF21) polynucleotide; thereby modulating a function of and/or the expression of the Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

E4. A method of modulating a function of and/or the expression of a Fibroblast growth factor 21 (FGF21) polynu-

cleotide in patient cells or tissues *in vivo* or *in vitro* comprising:

contacting said cells or tissues with at least one antisense oligonucleotide that targets a region of a natural antisense oligonucleotide of the Fibroblast growth factor 21 (FGF21) polynucleotide; thereby modulating a function of and/or the expression of the Fibroblast growth factor 21 (FGF21) polynucleotide in patient cells or tissues *in vivo* or *in vitro*.

5 E5. The method of E4, wherein a function of and/or the expression of the Fibroblast growth factor 21 (FGF21) is increased *in vivo* or *in vitro* with respect to a control.

E6. The method of E4, wherein the at least one antisense oligonucleotide targets a natural antisense sequence of a Fibroblast growth factor 21 (FGF21) polynucleotide.

10 E7. The method of E4, wherein the at least one antisense oligonucleotide targets a nucleic acid sequence comprising coding and/or non-coding nucleic acid sequences a Fibroblast growth factor 21 (FGF21) polynucleotide.

E8. The method of E4, wherein the at least one antisense oligonucleotide targets overlapping and/or nonoverlapping sequences of a Fibroblast growth factor 21 (FGF21) polynucleotide.

15 E9. The method of E4, wherein the at least one antisense oligonucleotide comprises one or more modifications selected from: at least one modified sugar moiety, at least one modified internucleotide linkage, at least one modified nucleotide, and combinations thereof.

E10. The method of E9, wherein the one or more modifications comprise at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and combinations thereof.

20 E11. The method of E9, wherein the one or more modifications comprise at least one modified internucleotide linkage selected from: a phosphorothioate, 2'-O-methoxyethyl (MOE), 2'-fluoro, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

25 E12. The method of E9, wherein the one or more modifications comprise at least one modified nucleotide selected from: a peptide nucleic acid (PNA), a locked nucleic acid (LNA), an arabino-nucleic acid (FANA), an analogue, a derivative, and combinations thereof.

E13. The method of E1, wherein the at least one oligonucleotide comprises at least one oligonucleotide sequences set forth as SEQ ID NOS: 3 to 9.

E14. A method of modulating a function of and/or the expression of a Fibroblast growth factor 21 (FGF21) gene in mammalian cells or tissues *in vivo* or *in vitro* comprising:

30 contacting said cells or tissues with at least one short interfering RNA (siRNA) oligonucleotide 5 to 30 nucleotides in length, said at least one siRNA oligonucleotide being specific for an antisense polynucleotide of a Fibroblast growth factor 21 (FGF21) polynucleotide, wherein said at least one siRNA oligonucleotide has at least 50% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense nucleic acid molecule of the Fibroblast growth factor 21 (FGF21) polynucleotide; and, modulating a function of and/or the expression of Fibroblast growth factor 21 (FGF21) in mammalian cells or tissues *in vivo* or *in vitro*.

35 E15. The method of E14, wherein said oligonucleotide has at least 80% sequence identity to a sequence of at least about five consecutive nucleic acids that is complementary to the antisense and/or sense nucleic acid molecule of the Fibroblast growth factor 21 (FGF21) polynucleotide.

40 E16. A method of modulating a function of and/or the expression of Fibroblast growth factor 21 (FGF21) in mammalian cells or tissues *in vivo* or *in vitro* comprising:

45 contacting said cells or tissues with at least one antisense oligonucleotide of about 5 to 30 nucleotides in length specific for noncoding and/or coding sequences of a sense and/or natural antisense strand of a Fibroblast growth factor 21 (FGF21) polynucleotide wherein said at least one antisense oligonucleotide has at least 50% sequence identity to at least one nucleic acid sequence set forth as SEQ ID NOS: 1 and 2; and, modulating the function and/or expression of the Fibroblast growth factor 21 (FGF21) in mammalian cells or tissues *in vivo* or *in vitro*.

50 E17. A synthetic, modified oligonucleotide comprising at least one modification wherein the at least one modification is selected from: at least one modified sugar moiety; at least one modified internucleotide linkage; at least one modified nucleotide, and combinations thereof; wherein said oligonucleotide is an antisense compound which hybridizes to and modulates the function and/or expression of a Fibroblast growth factor 21 (FGF21) gene *in vivo* or *in vitro* as compared to a normal control.

E18. The oligonucleotide of E17, wherein the at least one modification comprises an internucleotide linkage selected from the group consisting of phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

55 E19. The oligonucleotide of E17, wherein said oligonucleotide comprises at least one phosphorothioate internucleotide linkage.

E20. The oligonucleotide of E17, wherein said oligonucleotide comprises a backbone of phosphorothioate internucleotide linkages.

E21. The oligonucleotide of E17, wherein the oligonucleotide comprises at least one modified nucleotide, said modified nucleotide selected from: a peptide nucleic acid, a locked nucleic acid (LNA), analogue, derivative, and a combination thereof.

5 E22. The oligonucleotide of E17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: phosphorothioate, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and a combination thereof.

E23. The oligonucleotide of E17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified nucleotides selected from: peptide nucleic acids, locked nucleic acids (LNA), analogues, derivatives, and a combination thereof.

10 E24. The oligonucleotide of E17, wherein the oligonucleotide comprises at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

15 E25. The oligonucleotide of E17, wherein the oligonucleotide comprises a plurality of modifications, wherein said modifications comprise modified sugar moieties selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and a combination thereof.

E26. The oligonucleotide of E17, wherein the oligonucleotide is of at least about 5 to 30 nucleotides in length and hybridizes to an antisense and/or sense strand of a Fibroblast growth factor 21 (FGF21) polynucleotide wherein said oligonucleotide has at least about 20% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequences of the Fibroblast growth factor 21 (FGF21) polynucleotide.

20 E27. The oligonucleotide of E17, wherein the oligonucleotide has at least about 80% sequence identity to a complementary sequence of at least about five consecutive nucleic acids of the antisense and/or sense coding and/or noncoding nucleic acid sequence of the Fibroblast growth factor 21 (FGF21) polynucleotide.

25 E28. The oligonucleotide of E17, wherein said oligonucleotide hybridizes to and modulates expression and/or function of at least one Fibroblast growth factor 21 (FGF21) polynucleotide *in vivo* or *in vitro*, as compared to a normal control.

E29. The oligonucleotide of E17, wherein the oligonucleotide comprises the sequences set forth as SEQ ID NOS: 3 to 9.

30 E30. A composition comprising one or more oligonucleotides specific for one or more Fibroblast growth factor 21 (FGF21) polynucleotides, said polynucleotides comprising antisense sequences, complementary sequences, alleles, homologs, isoforms, variants, derivatives, mutants, fragments, or combinations thereof.

E31. The composition of E30, wherein the oligonucleotides have at least about 40% sequence identity as compared to any one of the nucleotide sequences set forth as SEQ ID NOS: 3 to 9.

35 E32. The composition of E30, wherein the oligonucleotides comprise nucleotide sequences set forth as SEQ ID NOS: 3 to 9.

E33. The composition of E32, wherein the oligonucleotides set forth as SEQ ID NOS: 3 to 9 comprise one or more modifications or substitutions.

40 E34. The composition of E33, wherein the one or more modifications are selected from: phosphorothioate, methylphosphonate, peptide nucleic acid, locked nucleic acid (LNA) molecules, and combinations thereof.

E35. A method of preventing or treating a disease associated with at least one Fibroblast growth factor 21 (FGF21) polynucleotide and/or at least one encoded product thereof, comprising:

45 administering to a patient a therapeutically effective dose of at least one antisense oligonucleotide that binds to a natural antisense sequence of said at least one Fibroblast growth factor 21 (FGF21) polynucleotide and modulates expression of said at least one Fibroblast growth factor 21 (FGF21) polynucleotide; thereby preventing or treating the disease associated with the at least one Fibroblast growth factor 21 (FGF21) polynucleotide and/or at least one encoded product thereof.

50 E36. The method of E35, wherein a disease associated with the at least one Fibroblast growth factor 21 (FGF21) polynucleotide is selected from: a disease or disorder associated with abnormal function and/or expression of FGF21, a metabolic disease or disorder (e.g., diabetes, obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, hypertension, hepatosteatosis such as non-alcoholic steatohepatitis (NASH) etc.), cancer, a disease or disorder associated with impaired lipid metabolism a disease or disorder associated with impaired renal function, a disease or disorder associated with impaired hepatic function, abnormal cell proliferation, a vascular disease or disorder (e.g., coronary artery disease, peripheral artery disease, atherosclerosis, abdominal aortic aneurysm, a blood clot, deep vein thrombosis, venous stasis disease, phlebitis, varicose veins etc.), angiogenesis, atherosclerosis, a cardiovascular disease or disorder, a disease or disorder associated with impaired blood vessel formation, a disease or disorder associated with impaired cell signaling, a disease or disorder associated with impaired kinase activity, a disease or disorder associated with impaired uptake of glucose into adipocytes, a disease or disorder associated with impaired inter-

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actions between FGF21 and one or both of FGFR-1 or FGFR-2, a disease or disorder associated with impaired phosphorylation of FGFR- 1 or FGFR-2 protein, and apoptosis.

E37. A method of identifying and selecting at least one oligonucleotide for in vivo administration comprising: selecting a target polynucleotide associated with a disease state; identifying at least one oligonucleotide comprising at least five consecutive nucleotides which are complementary to the selected target polynucleotide or to a polynucleotide that is antisense to the selected target polynucleotide; measuring the thermal melting point of a hybrid of an antisense oligonucleotide and the target polynucleotide or the polynucleotide that is antisense to the selected target polynucleotide under stringent hybridization conditions: and selecting at least one oligonucleotide for in vivo administration based on the information obtained.

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 30 <210> 9  
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 <213> Artificial Sequence  
  
 35 <220>  
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**Claims**

- 45 1. An oligonucleotide which targets a Fibroblast growth factor 21 (FGF21) natural antisense transcript for use as a therapeutic compound, wherein the natural antisense transcript has the nucleic acid sequence as set forth in SEQ ID NO: 2, wherein the oligonucleotide is single stranded, and wherein the oligonucleotide increases the expression of Fibroblast growth factor 21 (FGF21).
- 50 2. An oligonucleotide which targets a Fibroblast growth factor 21 (FGF21) natural antisense transcript for use in the prevention or treatment of a Fibroblast growth factor 21 (FGF21) associated disease or disorder, wherein the natural antisense transcript has the nucleic acid sequence as set forth in SEQ ID NO: 2, wherein the oligonucleotide is single stranded, and wherein the oligonucleotide increases the expression of Fibroblast growth factor 21 (FGF21).
- 55 3. Use of an oligonucleotide which targets a Fibroblast growth factor 21 (FGF21) natural antisense transcript for the manufacture of a medicament for the prevention of treatment of a Fibroblast growth factor 21 (FGF21) associated disease or disorder, wherein the natural antisense transcript has the nucleic acid sequence as set forth in SEQ ID NO: 2, wherein the oligonucleotide is single stranded, and wherein said oligonucleotide increases the expression of Fibroblast growth factor 21 (FGF21).

4. Use according to Claim 3, or an oligonucleotide for use according to Claim 2 wherein the disease or disorder is selected from the group consisting of a disease or disorder associated with abnormal function and/or expression of FGF21, a metabolic disease or disorder (e.g., diabetes, obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, hypertension, hepatosteatosis such as non-alcoholic steatohepatitis (NASH) etc.), a disease or disorder associated with impaired lipid metabolism, a disease or disorder associated with impaired renal function, a disease or disorder associated with impaired hepatic function, abnormal cell proliferation, a vascular disease or disorder (e.g., coronary artery disease, peripheral artery disease, atherosclerosis, abdominal aortic aneurysm, a blood clot, deep vein thrombosis, venous stasis disease, phlebitis, varicose veins etc.), angiogenesis, atherosclerosis, a cardiovascular disease or disorder, a disease or disorder associated with impaired blood vessel formation, a disease or disorder associated with impaired cell signaling, a disease or disorder associated with impaired kinase activity, a disease or disorder associated with impaired uptake of glucose into adipocytes, a disease or disorder associated with impaired interactions between FGF21 and one or both of FGFR-1 or FGFR-2, a disease or disorder associated with impaired phosphorylation of FGFR-1 or FGFR-2 protein, and apoptosis.
5. An *in vitro* method of increasing the expression of Fibroblast growth factor 21 (FGF21) patient cells or tissues comprising: contacting said cells or tissues with an oligonucleotide which targets a Fibroblast growth factor 21 (FGF21) natural antisense transcript, wherein the natural antisense transcript has the nucleic acid sequence as set forth in SEQ ID NO: 2, and wherein the oligonucleotide is single stranded; thereby increasing the expression of Fibroblast growth factor 21 (FGF21).
6. Use according to any one of Claims 3 or 4, or an oligonucleotide for use according to any one of Claims 1, 2 or 4, or a method according to Claim 5, wherein the expression of Fibroblast growth factor 21 (FGF21) is increased by at least 10%.
7. Use according to any one of Claims 3, 4 or 6, or an oligonucleotide for use according to any one of Claims 1, 2, 4 or 6, or a method according to any one of Claims 5 or 6, wherein the oligonucleotide further comprises one or more modifications comprising:
- at least one modified internucleoside linkage selected from: a phosphorothioate, 2'-O-methoxyethyl (MOE), 2'-fluoro, alkylphosphonate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof;
  - at least one modified nucleotide selected from: a peptide nucleic acid (PNA), a locked nucleic acid (LNA), an arabino-nucleic acid (FANA), an analogue, a derivative, and combinations thereof; or
  - at least one modified sugar moiety selected from: a 2'-O-methoxyethyl modified sugar moiety, a 2'-methoxy modified sugar moiety, a 2'-O-alkyl modified sugar moiety, a bicyclic sugar moiety, and combinations thereof.
8. An oligonucleotide having the features of an oligonucleotide defined in any one of Claims 1 to 7, wherein the oligonucleotide does not comprise SEQ ID NO: 8.
9. An oligonucleotide according to Claim 8, wherein the oligonucleotide is between 10 to 30 nucleotides in length.
10. An oligonucleotide according to Claim 8 or 9, wherein the oligonucleotide has at least 90% sequence identity to a complement of a Fibroblast growth factor 21 (FGF21) natural antisense transcript.
11. A pharmaceutical composition comprising at least one oligonucleotide according to any one of Claims 8 to 10 and a pharmaceutically acceptable excipient.

FIGURE 1

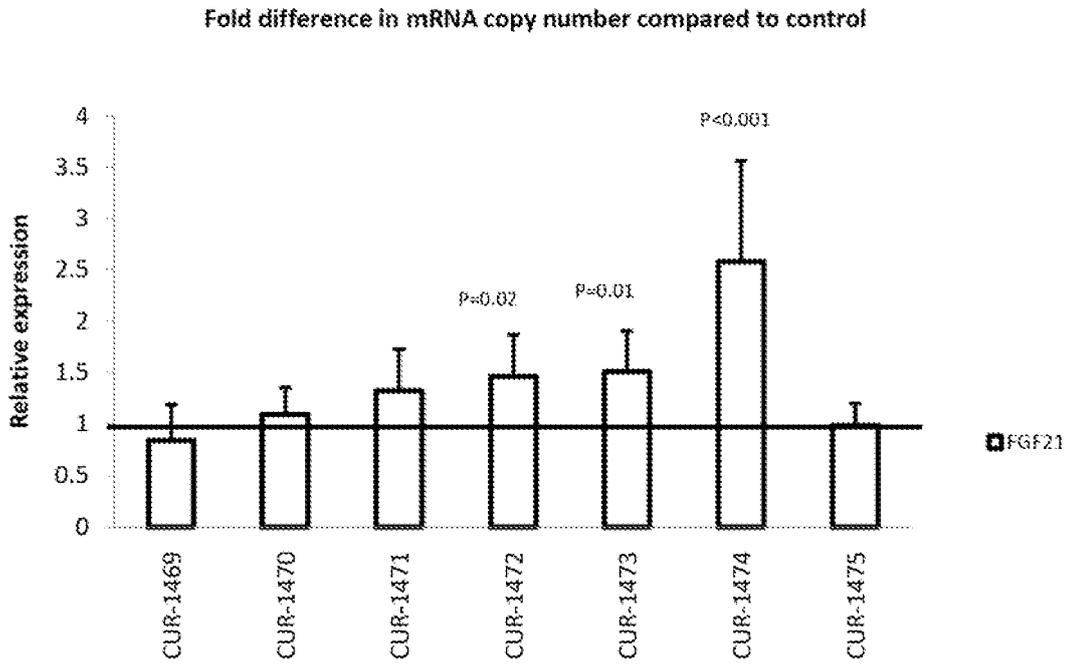
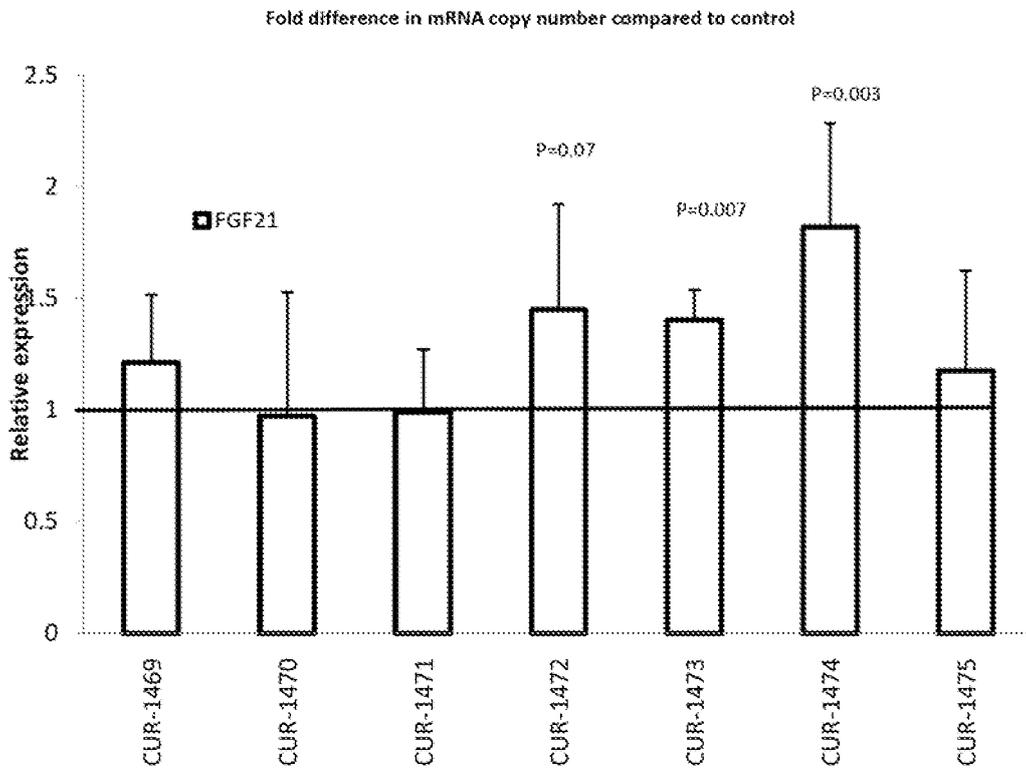


FIGURE 2





EUROPEAN SEARCH REPORT

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Y	* the whole document * * page 288, right-hand column, paragraph 1 *	1-4,6-11	
X	----- THOMAS M. MOEHLER ET AL: "Involvement of [alpha] 1-2-fucosyltransferase I (FUT1) and surface-expressed lewis y (CD174) in first endothelial cell-cell contacts during angiogenesis", JOURNAL OF CELLULAR PHYSIOLOGY, vol. 215, no. 1, 1 April 2008 (2008-04-01), pages 27-36, XP055012437, ISSN: 0021-9541, DOI: 10.1002/jcp.21285	1-4,8-11	
Y	* the whole document * * page 28, right-hand column, last paragraph *	1-4,6-11	TECHNICAL FIELDS SEARCHED (IPC) A61K
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The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 27 May 2019	Examiner Orlando, Michele
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	

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Application Number  
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Y	KODA Y ET AL: "STRUCTURE AND EXPRESSION OF H-TYPE GDP-L-FUCOSE:BETA-D-GALACTOSIDE 2-ALPHA-L-FUCOSYLTRANSFERASE GENE (FUT1) TWO TRANSCRIPTION START SITES AND ALTERNATIVE SPLICING GENERATE SEVERAL FORMS OF FUT1 MRNA", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 272, no. 11, 14 March 1997 (1997-03-14), pages 7501-7505, XP002919590, ISSN: 0021-9258, DOI: 10.1074/JBC.272.11.7501 * the whole document *	1-4,6-11	
A,P	DOMENICO PALUMBERI ET AL: "RNA-mediated gene silencing of FUT1 and FUT2 influences expression and activities of bovine and human fucosylated nucleolin and inhibits cell adhesion and proliferation", JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 111, no. 1, 1 September 2010 (2010-09-01), pages 229-238, XP055070856, ISSN: 0730-2312, DOI: 10.1002/jcb.22692	1-11	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC)
Place of search Munich		Date of completion of the search 27 May 2019	Examiner Orlando, Michele
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	

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